

549,951

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
7 October 2004 (07.10.2004)

PCT

(10) International Publication Number
WO 2004/084809 A2

- (51) International Patent Classification⁷: **A61K**
- (21) International Application Number:
PCT/US2004/008108
- (22) International Filing Date: 16 March 2004 (16.03.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/456,136 20 March 2003 (20.03.2003) US
60/505,119 23 September 2003 (23.09.2003) US
- (71) Applicants (for all designated States except US):
NEURONZ LIMITED [NZ/NZ]; Level 3, 2-6 Park Avenue, Grafton, Auckland 1030 (NZ). **NEURONZ BIOSCIENCES, INC.** [US/US]; 2711 Centerville Road - Suite 400, Wilmington, De 19801 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **HARRIS, Paul, W.**, R. [NZ/NZ]; 111 Christian Road, Swansan, Auckland 1030 (NZ). **BRIMBLE, Margaret, Anne** [NZ/NZ]; 11 A Torrance Street, Epsom, Auckland 1003 (NZ).
- (74) Agent: **BORSON, D., Benjamin**; Fliesler Meyer LLP, Four Embarcadero Center, Fourth Floor, San Francisco, CA 94111-4156 (US).

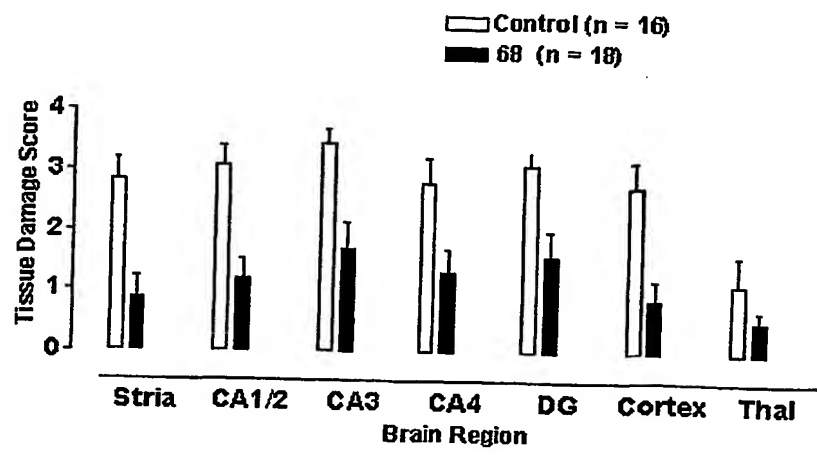
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NEUROPROTECTIVE MACROCYCLIC COMPOUNDS AND METHODS FOR THEIR USE

100 pM 68 given i.c.v. 2 h after HI injury



(57) Abstract: Embodiments of this invention provide novel peptidomimetics that contain a macrocycle. Such compounds are neuroprotective and have utility as therapeutic agents for treatment of diseases, injuries and other conditions characterised by neuronal degeneration and/or death. Compounds are also useful for manufacture of medicaments useful for treatment of such conditions.

WO 2004/084809 A2

NEUROPROTECTIVE MACROCYCLIC COMPOUNDS AND METHODS FOR THEIR USE

Related Application

This application claims priority to U.S. Provisional patent applications
5 Serial No: 60/456,136, filed March 20, 2003 and Serial No: 60/505,119, filed
September 23, 2003, both herein incorporated fully by reference.

FIELD OF THE INVENTION

The present invention relates to novel peptidomimetics containing a
macrocycle and methods of their use. This invention also relates to the
10 neuroprotective activity of such compounds. More particularly, this invention
relates to the use of these compounds and pharmaceutical compositions thereof
in the treatment of diseases and conditions characterised by neuronal
degeneration and/or death.

15 BACKGROUND

The degeneration and/or death of cells in the nervous system is a major
factor in many diseases and medical conditions. Such diseases and conditions
include traumatic brain injuries, traumatic spinal cord injuries, stroke, hypoxia
or ischemia related to decreased neural perfusion secondary to cardiac arterial
20 bypass graft surgery (CABG), Parkinson's disease, Alzheimer's disease,
multiple sclerosis, amyotrophic lateral sclerosis and other neurodegenerative
diseases. It is of interest to prevent or decrease such cell death and degeneration
and to minimize the loss of neural function.

Certain compounds are useful as neuroprotective agents. One such
25 compound is insulin-like growth factor 1 (IGF-1) (Scheepens *et al*,
WO00/13650). IGF-1 is a naturally occurring peptide that can decrease the
binding of glutamate to the glutamate receptors of neurons (Bourguignon, US
Patent No. 5,804,550). IGF-1 can also decrease neuronal degradation caused by
damage and disease. IGF-1 is cleaved by proteolysis *in vivo* to give des₁₋₃ IGF-

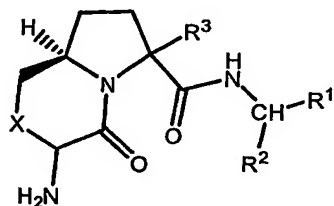
1 and the N-terminal tripeptide Gly-Pro-Glu (GPE). GPE and analogues have been found to be neuroprotective (Gluckman *et al*, US Patent No. 6,187,906 incorporated herein by reference).

5 However, such peptides may not be ideal for the treatment of neural death and degeneration especially in conditions in which they are rapidly metabolised *in vivo*. There is a need for compounds that have neuroprotective and neuroregenerative properties and that are more metabolically stable, especially to enzymatic degradation.

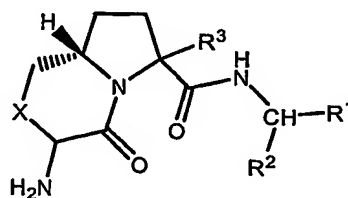
10 The use of peptidomimetics to mimic the behaviour of biologically active peptides is common in the pursuit of a drug candidate. Peptidomimetics that are more metabolically stable and protease resistant than peptides are desirable, and they often adopt well-defined conformations. Cyclic structures can provide a rigid geometry that can be used to probe the bioactive conformation of a given peptide. This rigidity conformationally restricts the
15 molecule and thus provides a method to design molecules with enhanced biological activity.

SUMMARY

20 Aspects of this invention include novel cyclic peptidomimetics that can mimic certain properties of the tripeptide Gly-Pro-Glu (GPE). Certain aspects of the invention include molecules having the structural formulae and substituents described below:



25 **Formula 1**



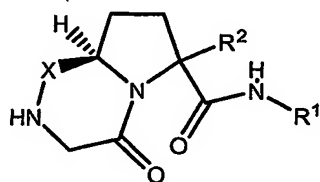
Formula 2

In certain embodiments, compounds of Formula 1 and Formula 2 include substituents where:

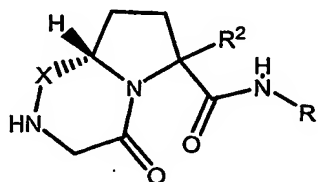
R^1 and R^2 are independently selected from the group consisting of -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

R^3 is selected from the group consisting of -H, -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₃.



Formula 3



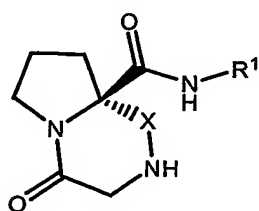
Formula 4

In other embodiments, compounds of Formula 3 and Formula 4 include substituents where:

R^1 and R^2 are independently selected from the group consisting of -H, -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R',

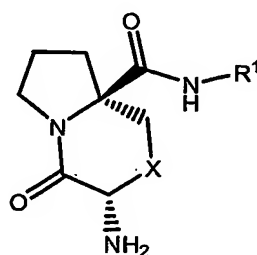
trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is
 5 independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₄.



10

Formula 5

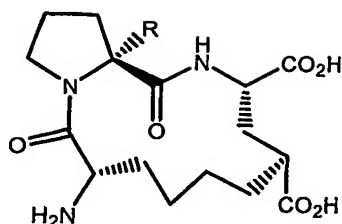


Formula 6

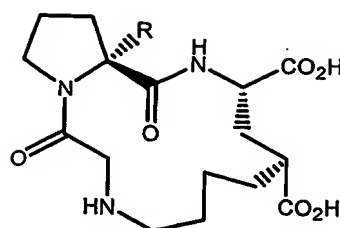
In other embodiments, compounds of Formula 5 and Formula 6 include substituents where:

R is independently selected from the group consisting of -H, -OR', -SR',
 15 -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is
 20 independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₄.



Formula 7



Formula 8

In other embodiments, compounds of Formula 7 and Formula 8 include
 5 substituents where:

R is independently selected from the group consisting of -H, -OR', -SR',
 -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R',
 trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted
 heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl,
 10 substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted
 arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is
 independently selected from the group consisting of -H, alkyl, heteroalkyl,
 alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl.

Other aspects of the invention provide pharmaceutically acceptable salts
 15 of the compounds described in formulas 1-8.

In still other aspects, this invention provides pharmaceutical
 compositions comprising a pharmaceutically acceptable excipient and a
 therapeutically effective amount of at least one compound of this invention.
 These compositions find use as anti-apoptotic and anti-necrotic agents and for
 20 other conditions involving neural degeneration or injury.

In yet further aspects, this invention provides a method of treating an
 animal having a disease or injury capable of treatment by administration of a
 suitable compound, comprising administration to that animal of at least one
 compound of this invention, optionally in conjunction with at least one other
 25 conventional therapeutic agent for the disease being treated.

In still further aspects the animal to be treated is a human.

In still further aspects, this invention provides methods of preparing the compounds of Formulas 1-8 of this invention.

In yet other aspects, this invention provides methods of synthesising, formulating and preparing pharmaceutical preparations comprising compounds of Formulas 1-8 of this invention.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graph showing effects of (2S, 3'S, 8'R, 11'S) 2-{[(3'-Amino-1'-aza—2'-oxobicyclo[6.3.0]undecyl)-11'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 48 on neuronal survival in animals following excitotoxic oxidative stress.

FIG. 2 is a graph showing effects of (2S, 9'R, 12'S)-2-{[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate 68 on neuronal survival in animals following excitotoxic oxidative stress.

FIG. 3 is a graph showing the neuroprotective effects of (2S, 9'R, 12'S)-2-{[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate 68 in a global model of brain ischaemia.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Alkenyl" refers to an unsaturated branched, straight chain or cyclic hydrocarbon radical having at least one carbon-carbon double bond. The radical can be in either the *cis* or *trans* conformation about the double bond(s). Exemplary alkenyl groups include allyl, ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, cyclopentenyl and the like. In some embodiments the

alkenyl groups are (C₂-C₆) alkenyl and in other embodiments allyl can be particularly useful.

“Alkyl” refers to a saturated branched, straight chain or cyclic hydrocarbon radical. Exemplary alkyl groups include methyl, ethyl, isopropyl, cyclopropyl, *tert*-butyl, cyclopropylmethyl, hexyl and the like. In some embodiments the alkyl groups are (C₁-C₆) alkyl.

“Alkynyl” refers to an unsaturated branched, straight chain or cyclic hydrocarbon radical having at least one carbon-carbon triple bond. Exemplary alkynyl groups include ethynyl, propynyl, butynyl, isobutynyl and the like. In some embodiments the alkynyl group is (C₂-C₆) alkynyl.

“Aryl” refers to an unsaturated cyclic hydrocarbon radical with a conjugated π electron system. Exemplary aryl groups include phenyl, naphthyl and the like. In some embodiments the aryl group is (C₅-C₂₀) aryl.

“Arylalkyl” refers to a straight chain alkyl, alkenyl or alkynyl group wherein one of the hydrogen atoms bonded to the terminal carbon is replaced with an aryl group. Exemplary arylalkyl groups include benzyl, naphthylmethyl, benzylidene and the like.

A “growth factor” refers to an extracellular polypeptide-signalling molecule that stimulates a cell to grow or proliferate.

“Heteroalkyl” refers to an alkyl moiety wherein one or more carbon atoms are replaced with another atom such as N, P, O, S etc. Exemplary heteroalkyl groups include pyrrolidine, morpholine, piperidine, piperazine, imidazolidine, pyrazolidine, tetrahydrofuran, (C₁-C₁₀) substituted amines, (C₂-C₆) thioethers and the like.

“Heteroaryl” refers to an aryl moiety wherein one or more carbon atoms are replaced with another atom such as N, P, O, S etc. Exemplary heteroaryl groups include carbazole, furan, imidazole, indazole, indole, isoquinoline,

purine, pyrazine, pyrazole, pyridazine, pyridine, pyrrole, thiazole, thiophene, triazole and the like.

“Injury” includes any acute or chronic damage of an animal that results in degeneration or death of cells in the nervous system or results in loss of function. Such cells include neuronal cells and non-neuronal cells. Injury includes stroke, non-hemorrhagic stroke, traumatic brain injury, perinatal asphyxia associated with fetal distress such as following abruption, cord occlusion or associated with intrauterine growth retardation, perinatal asphyxia associated with failure of adequate resuscitation or respiration, severe CNS insults associated with near miss drowning, near miss cot death, carbon monoxide inhalation, ammonia or other gaseous intoxication, cardiac arrest, coma, meningitis, hypoglycemia and status epilepticus, episodes of cerebral asphyxia associated with coronary bypass surgery, hypotensive episodes and hypertensive crises, and cerebral trauma. It is to be understood that the above examples are by way of illustration only, and are not intended to be a complete listing of injuries capable of being treated by the compounds and methods of this invention.

A “pharmaceutically acceptable excipient” refers to an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

A “pharmaceutically acceptable salt” refers to a salt that is pharmaceutically acceptable and has the desired pharmacological properties. Such salts include salts that can be formed where acidic protons present in the compounds are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, e.g. sodium and potassium, magnesium, calcium, and aluminium. Suitable organic salts include those formed with organic bases such as the amine bases e.g. ethanolamine,

diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (e.g. hydrochloric and hydrobromic acids) and organic acids (e.g. acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic acids such as methanesulfonic acid and benzenesulfonic acid). When there are two acidic groups present, a pharmaceutically acceptable salt can be a mono-acid mono-salt or a di-salt; and similarly where there are more than two acidic groups present, some or all of such groups can be salified.

A "protecting group" has the meaning conventionally associated with it in organic synthesis, i.e. a group that selectively blocks one or more reactive sites in a multifunctional compound such that a chemical reaction can be carried out selectively on another unprotected reactive site and such that the group can readily be removed after the selective reaction is complete.

"Substituted" refers to where one or more of the hydrogen atoms on an alkyl, heteroalkyl, alkenyl, alkynyl, aryl, heteroaryl or arylalkyl radical are independently replaced with another substituent. Substituents include -R', -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', -NR'-C(NR')-OR', -NR'-C(NR')-SR', NR'-C(NR')-NR'R', trihalomethyl and halogen where each R' is independently -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl or heteroarylalkyl.

A "therapeutically effective amount" means the amount that, when administered to an animal for treating a disease, is sufficient to effect treatment for that disease; that is, an amount that decreases adverse symptoms or findings, promotes desirable symptoms or findings, and/or treats an underlying disorder, and/or is curative.

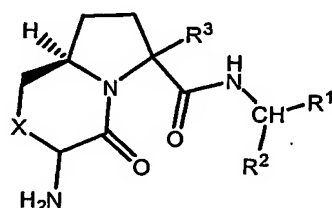
"Treating" or "treatment" of a disease includes preventing the disease from occurring in an animal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment), inhibiting the disease (slowing or arresting its development), providing relief

from the symptoms or side-effects of the disease (including palliative treatment), and relieving the disease (causing regression of the disease).

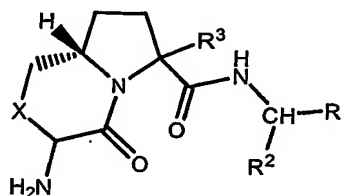
Implicit hydrogen atoms (such as the hydrogens on the pyrrole ring, etc.) are omitted from the formulae for clarity, but should be understood to be present.

Compounds of the Invention

Certain aspects of the invention provide molecules having the structural formulae and substituents described below:



Formula 1



Formula 2

In certain embodiments, compounds of Formula 1 and Formula 2 include substituents where:

R^1 and R^2 are independently selected from the group consisting of $-OR'$, $-SR'$, $-NR'R'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R'$, $-C(NR')NR'R'$, trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of $-H$, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

R^3 is selected from the group consisting of $-H$, $-OR'$, $-SR'$, $-NR'R'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R'$, $-C(NR')NR'R'$, trihalomethyl,

halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently
 5 selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₃.

In further embodiments of the invention the compounds are compounds of Formula 1 where R¹ = -COOH; R² = -(CH₂)₂-COOH; R³ = H; X = -(CH₂)₃-

10 In other embodiments of the invention the compounds are compounds of Formula 1 where R¹ = -COOH; R² = -(CH₂)₂-COOH; R³ = CH₃; X = -(CH₂)₃-

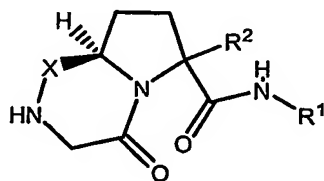
In still other embodiments of the invention the compounds are compounds of formula 1 where R¹ = -COOH; R² = -(CH₂)₂-COOH; R³ = allyl; X = -(CH₂)₃-

15 In yet further embodiments of the invention the compounds are compounds of formula 1 where R¹ = -COOH; R² = -(CH₂)₂-CH₃; R³ = H; X = -(CH₂)₃-

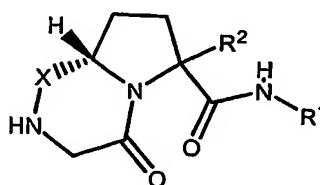
In still other embodiments of the invention the compounds are compounds of formula 1 where R¹ = -COOH; R² = -(CH₂)₂-COOH; R³ = H; X =
 20 -(CH₂)₂-

In further embodiments of the invention the compounds are compounds of formula 1 where R¹ = -COOH; R² = -(CH₂)₂-COOH; R³ = H; X = -CH₂-CH=CH-, (where the CH₂ of X is adjacent to the carbon attached to the NH₂ group).

25



Formula 3



Formula 4

In other embodiments, compounds of Formula 3 and Formula 4 include substituents where:

R^1 and R^2 are independently selected from the group consisting of -H, -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₄.

In further embodiments of the invention the compounds are compounds of formula 3 where $R^1 = -CH(COOH)-(CH_2)_2-COOH$; $R^2 = H$; $X = -(CH_2)_4-$

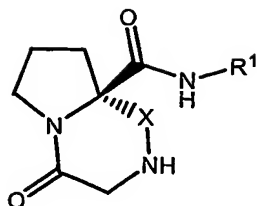
In still other embodiments of the invention the compounds are compounds of formula 3 where $R^1 = -CH(COOH)-(CH_2)_2-COOH$; $R^2 = CH_3$; $X = -(CH_2)_4-$

In yet further embodiments of the invention the compounds are compounds of formula 3 where $R^1 = -CH(COOH)-(CH_2)_2-COOH$; $R^2 = \text{allyl}$; $X = -(CH_2)_4-$

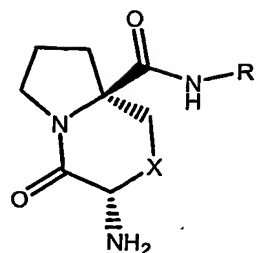
In further embodiments of the invention the compounds are compounds of formula 3 where $R^1 = -CH(COOH)-(CH_2)_2-CH_3$; $R^2 = H$; $X = -(CH_2)_4-$

In other embodiments of the invention the compounds are compounds of formula 3 where $R^1 = -CH(COOH)-(CH_2)_2-COOH$; $R^2 = H$; $X = -(CH_2)_3-$

In still other embodiments of the invention the compounds are compounds of formula 3 where $R^1 = -CH(COOH)-(CH_2)_2-COOH$; $R^2 = H$; $X = -CH_2-CH=CH-CH_2-$



Formula 5

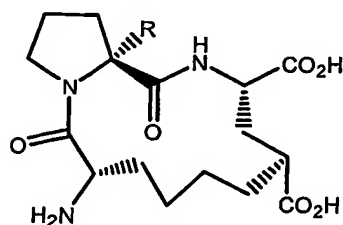


Formula 6

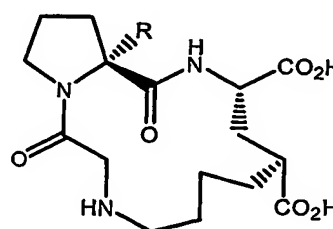
In other embodiments, compounds of Formula 5 and Formula 6 include substituents where:

R is independently selected from the group consisting of -H, -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₄.



Formula 7



Formula 8

In other embodiments, compounds of Formula 7 and Formula 8 include substituents where:

R is independently selected from the group consisting of -H, -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl.

In further embodiments of the invention the compounds are compounds of formula 7 where R=H.

In yet other embodiments of the invention the compounds are compounds of formula 7 where R=CH₃.

In yet further embodiment of the invention the compounds are compounds of formula 7 where R=allyl.

Other aspects of the invention provide pharmaceutically acceptable salts of the compounds described in Formulas 1-8.

In still other aspects, this invention provides pharmaceutical compositions comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of at least one compound of this invention. These compositions find use as anti-apoptotic and anti-necrotic agents and for other conditions involving neural degeneration or injury.

In further aspects, this invention provides methods of treating an animal having a disease or injury capable of treatment by administration of a suitable compound of Formulas 1-8, comprising administration to that animal of at least one compound of this invention, optionally in conjunction with at least one other conventional therapeutic agent for the disease being treated.

In yet further aspects, the animal to be treated is a human.

In still further aspects, this invention provides methods of synthesizing, formulating and preparing pharmaceutical preparations comprising compounds of Formulas 1-8 of this invention.

Those with skill in the art will appreciate that the above structural formulae contain chiral centres, the number of which will depend on the different substituents. The chirality is only indicated for some centres. The chirality can be either R or S at each centre. The formulae drawings represent
5 only one of the possible tautomeric, conformational isomeric or enantiomeric forms, it should be understood that the invention encompasses any tautomeric, conformational isomeric or enantiomeric forms which exhibit biological or pharmacological activity as described herein.

10 Pharmacology and Utility

Certain aspects of this invention include the use of compounds of the invention in treatment or prevention of cell damage, degeneration and/or death in mammals in response to injury or disease. Some embodiments comprise delivering a composition containing a compound of the invention to an animal
15 suffering from neural degeneration, and in some cases, conditions involving apoptotic and necrotic cell death. In some embodiments, compositions are desirable to treat an injury or disease of the CNS affecting or liable to affect brain cells. Compositions are provided that can also include one or more other agents that promote neural regeneration, decrease cell degeneration or death, or
20 are neuroprotective.

Such other agents can be selected from the group consisting of for example, growth factors and associated derivatives, e.g., insulin-like growth factor-I [IGF-I], insulin-like growth factor-II [IGF-II], the tripeptide GPE, transforming growth factor- β 1, activin, growth hormone, nerve growth factor,
25 growth hormone binding protein, and/or IGF-binding proteins.

Other aspects of the invention include compositions and methods of promoting fasciculation of axons. By promoting formation of nerve bundles, compounds of the invention can be useful in treating conditions in which nerve processes (axons and/or dendrites) have become severed, such as in sharp force

injuries, local areas of necrosis or disease, or other localized injuries to nerve processes.

5 In yet other embodiments, compositions and methods to treat or prevent cell damage and death in response to injury and disease, including CNS injury and disease, comprise administration of a therapeutic amount of a compound of the invention alone or in combination with other agents, after the insult. These embodiments can be particularly desirable in situations of unexpected injury, such as in cardiac arrest, trauma such as head injuries caused by automobile accidents, head wounds and the like.

10 In still further embodiments, compounds of the invention can be used either alone or in combination with other agents to prevent adverse effects of planned brain injury. Such conditions include CABG or other planned surgeries such as brain surgery, vascular surgery or other interventions that can lead to decreased perfusion of the nervous system. By treating an animal, such as a
15 human being, in advance and/or simultaneously and/or after the surgery, adverse neurological effects may be ameliorated.

As indicated above, the present invention is broadly based upon the applicant's finding that compounds of the invention can protect cells, particularly nerve cells, against damage, loss of neurites, and/or apoptotic or
20 necrotic cell death.

It is herein demonstrated that compounds of the invention exhibit neuroprotection in cell culture models of neurodegenerative disease and can therefore be an effective addition or alternative to conventional therapies for neural degeneration.

25 Although the mechanism of the protective effects is not known, one possible mechanism involves protecting cells from apoptotic and necrotic cell death. However, regardless of the mechanism of action, compounds of the invention can be used as an effective therapy for a variety of neurological diseases, including hypoxia, ischemia and neurotoxin-induced nerve damage.

Moreover, compounds of the invention can be used in the absence of any particular neurological deficit to promote neurite outgrowth and fasciculation of nerves. Thus, in situations in which cell death is not necessarily associated with the neurological disorder (e.g., axonal damage such as caused by spinal cord injury), administration of compounds of the invention may be an effective way of promoting neurite regeneration.

Therapeutic Applications

Compositions and methods of the invention find use in the treatment of animals, such as human patients, suffering from neural injury or disease. Still more generally, the compositions and methods of the invention find use in the treatment of mammals, such as human patients, suffering from nerve damage or potential apoptotic and/or necrotic cell death, due to injuries and diseases.

Specific conditions and diseases characterised by neuronal degeneration, apoptosis and/or necrosis include but are not limited to Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, peripheral neuropathy, Creutzfeldt-Jakob disease, AIDS dementia, progressive supranuclear palsy, myelinopathia centralis diffusa (vanishing white matter disease), chronic neurodegenerative disease, Huntington's disease, stroke, ischemic injury, hypoxic injury, reperfusion injury, head injury, CNS trauma, epilepsy, cerebral ischemia, glaucoma, retinal disorders, optic neuropathy, optic neuritis, Down's syndrome, encephalomyelitis, meningitis, panencephalitis, neuroblastoma, schizophrenia and depression. Each of the above conditions exhibits pathophysiological findings and symptoms that are mimicked by neurotoxicity associated with glutamate toxicity.

Still more generally, the invention has application in the induction of nerve bundle formation following insult in the form of trauma, toxin exposure, asphyxia or hypoxia-ischemia. Additionally, the invention has application in

the treatment or prevention of apoptosis in response to injury or disease in the form of cancers, viral infections, autoimmune diseases, neurological diseases and injuries and cardiovascular diseases.

5 Treatment can be given before an injury, for example, before elective surgery. Examples of relevant elective procedures include neural surgery, in which retraction of lobes of the brain can lead to cerebral oedema, or heart operations, such as valve replacement, in which inevitable small emboli are said to lead to detectable impairment of brain function in some 75% of cases.

10 **Determining Efficacy**

The anti-apoptotic and anti-necrotic activity of compounds of the invention can be measured by *in vivo* using cell counts by methods known to those skilled in the art including the methods of Klempt *et al* (Klempt *et al*, 1992, *Molecular Brain Research*: 13: 93-101), microscopic examinations of
15 morphology, cell counts of surviving and dead neurons stained with thionin/fuchsin and the like. Compounds of the invention can also be measured *in vitro* using mass spectroscopy, immunological, or chromatographic methods known in the art.

CNS damage can for example be measured clinically by the degree of
20 permanent neurological deficit cognitive function, and/or propensity to seizure disorders. Herein are disclosed histological techniques suitable for measuring effects *in vivo*.

The therapeutic ratio of a compound is understood to be the ratio of (1) the mean dose that causes adverse side effect over (2) the mean dose that causes
25 a desirable therapeutic effect. Thus, for compounds for which have therapeutic effects at relatively low doses and undesirable side effects at high doses, the therapeutic ratio is >1 . Therapeutic ratio can be determined, for example, by comparing the dose that produces significant weight loss (or other observable side-effect) divided by the dose that produces anti-apoptotic and anti-necrotic
30 activity in a suitable *in vivo* animal species such as the rat or mouse. Suitable

models include a hypoxic-ischemic injury (Sirimanne *et al*, 1994 *Journal of Neuroscience Methods*: 55: 7-14) and experimental immune encephalomyelitis (Mendel *et al.*, 1995 *Eur. J. Immunol.*: 25: 1951-1959).

5 **Pharmaceutical compositions and administration**

Compounds of the invention can be administered as part of a medicament or pharmaceutical preparation. This can involve combining a compound of the invention with any pharmaceutically appropriate carrier, adjuvant or excipient. The selection of the carrier, adjuvant or excipient will of course usually be dependent upon the route of administration to be employed.

In general, compounds of this invention will be administered in therapeutically effective amounts by any of the usual modes known in the art, either singly or in combination with other conventional therapeutic agents for the disease being treated. A therapeutically effective amount can vary widely depending on the disease or injury, its severity, the age and relative health of the animal being treated, the potency of the compound(s), and other factors. As anti-apoptotic and anti-necrotic agents, therapeutically effective amounts of compounds of this invention can range from 0.001 to 100 milligrams per kilogram mass of the animal, with lower doses such as 0.001 to 0.1 mg/kg being appropriate for administration through the cerebrospinal fluid, such as by intracerebroventricular administration, and higher doses such as 1 to 100 mg/kg being appropriate for administration by methods such as oral, systemic (e.g. transdermal), or parenteral (e.g. intravenous) administration. A person of ordinary skill in the art will be able without undue experimentation, having regard to that skill and this disclosure, to determine a therapeutically effective amount of a compound of this invention for a given disease or injury.

Compounds of the invention can be administered peripherally *via* any peripheral route known in the art. These can include parenteral routes for example injection into the peripheral circulation, subcutaneous, intraorbital, ophthalmic, intraspinal, intracisternal, topical, infusion (using e.g. slow release

devices or minipumps such as osmotic pumps or skin patches), implant, aerosol, inhalation, scarification, intraperitoneal, intracapsular, intramuscular, intranasal, oral, buccal, transdermal, pulmonary, rectal or vaginal. The compositions can be formulated for parenteral administration to humans or other mammals in
5 therapeutically effective amounts (e.g. amounts which eliminate or reduce the patient's pathological condition) to provide therapy for the neurological diseases described above.

Desirably, if possible, when administered as anti-apoptotic and anti-necrotic agents, compounds of this invention will be administered orally. The
10 amount of a compound of this invention in the composition can vary widely depending on the type of composition, size of a unit dosage, kind of excipients, and other factors well known to those of ordinary skill in the art. In general, the final composition can comprise from 0.0001 percent by weight (% w) to 10% w of the compound of this invention, preferably 0.001% w to 1% w, with the
15 remainder being the excipient or excipients.

Other convenient administration routes include subcutaneous injection (e.g. dissolved in a physiologically compatible carrier such as 0.9% sodium chloride) or direct administration to the CNS. Using stereotactic devices and accurate maps of an animal's CNS, a compound can be injected directly into a
20 site of neural damage. Such routes of administration can be especially desired in situations in which perfusion of that location is compromised either by decreased vascular perfusion or by decreased cerebral spinal fluid (CSF) flow to that area. Examples include administration by lateral cerebroventricular injection or through a surgically inserted shunt into the lateral cerebroventricle
25 of the brain of the patient, intravenously, direct injection into the desired location or other routes.

The effective amount of compound in the CNS can be increased by administration of a pro-drug form of a compound which comprises a compound of the invention and a carrier, where the carrier is joined to a compound of the

invention by a linkage which is susceptible to cleavage or digestion within the patient. Any suitable linkage can be employed which will be cleaved or digested following administration.

However, there is no intention on the part of the applicants to exclude
5 other forms of administration.

In further embodiments of the invention, restoring nerve function in an animal can comprise administering a therapeutic amount of a compound of the invention in combination with another neuroprotective agent, selected from, for example, growth factors and associated derivatives (insulin-like growth factor-I
10 [IGF-I], insulin-like growth factor-II [IGF-II], transforming growth factor- β 1, activin, growth hormone, nerve growth factor, growth hormone binding protein, IGF-binding proteins [especially IGFBP-3], basic fibroblast growth factor, acidic fibroblast growth factor, the hst/Kfgk gene product, FGF-3, FGF-4, FGF-6, keratinocyte growth factor, androgen-induced growth factor. Additional
15 members of the FGF family include, for example, int-2, fibroblast growth factor homologous factor-1 (FHF-1), FHF-2, FHF-3 and FHF-4, keratinocyte growth factor 2, glial-activating factor, FGF-10 and FGF-16, ciliary neurotrophic factor, brain derived growth factor, neurotrophin 3, neurotrophin 4, bone morphogenetic protein 2 [BMP-2], glial-cell line derived neurotrophic factor,
20 activity-dependant neurotrophic factor, cytokine leukaemia inhibiting factor, oncostatin M, interleukin), α -, β -, γ -, or consensus interferon, and TNF- α . Other forms of neuroprotective therapeutic agents include, for example, clomethiazole; kynurenic acid, Semax, tacrolimus, L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, adrenocorticotropin-(4-9) analogue
25 [ORG 2766] and dizolcipine [MK-801], selegiline; glutamate antagonists such as, NPS1506, GV1505260, MK-801, GV150526; AMPA antagonists such as 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX), LY303070 and LY300164; anti-inflammatory agents directed against the addressin MAdCAM-1 and/or its integrin α 4 receptors (α 4 β 1 and α 4 β 7), such as anti-
30 MAdCAM-1mAb MECA-367 (ATCC accession no. HB-9478).

The compound can be administered by a sustained-release system. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919; EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman
5 *et al.*, 1983, *Biopolymers*: 22: 547-56), poly(2-hydroxyethyl methacrylate) (Langer *et al.*, 1981, *J. Biomed. Mater. Res.*: 15: 267), ethylene vinyl acetate (Langer *et al.*, 1981, *J. Biomed. Mater. Res.*: 15: 267), or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include
10 a liposomally entrapped compound. Liposomes containing the compound are prepared by methods known per se: DE 3,218,121, EP 52,322, EP 36,676, EP 88,046, EP 143,949, EP 142,641, Japanese Pat. Appln. 83-118008, U.S. Pat. Nos. 4,485,045 and 4,544,545, and EP 102,324. Ordinarily, the liposomes are of the small (from or about 200 to 800 Angstroms) unilamellar type in which the
15 lipid content is greater than about 30 mol percent cholesterol, the selected proportion being adjusted for the most efficacious therapy.

For parenteral administration, in one embodiment the compound is formulated generally by mixing each at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a
20 pharmaceutically, or parenterally, acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.

Generally, the formulations are prepared by contacting the compound uniformly and intimately with liquid carriers or finely divided solid carriers or
25 both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, a buffered solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also
30 useful herein.

A carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; glycine; amino acids such as glutamic acid, aspartic acid, histidine, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, trehalose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counter-ions such as sodium; non-ionic surfactants such as polysorbates, poloxamers, or polyethylene glycol (PEG); and/or neutral salts, e.g., NaCl, KCl, MgCl₂, CaCl₂, etc.

A compound is typically formulated in such vehicles at a pH of from or about 4.5 to 8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of salts of the compound. The final preparation can be a stable liquid or lyophilized solid.

Formulations of the compound in pharmaceutical compositions can also include adjuvants. Typical adjuvants which can be incorporated into tablets, capsules, and the like are a binder such as acacia, corn starch, or gelatin; an excipient such as microcrystalline cellulose; a disintegrating agent like corn starch or alginic acid; a lubricant such as magnesium stearate; a sweetening agent such as sucrose or lactose; a flavouring agent such as peppermint, wintergreen, or cherry. When the dosage form is a capsule, in addition to the above materials, it can also contain a liquid carrier such as a fatty oil. Other materials of various types can be used as coatings or as modifiers of the physical form of the dosage unit. A syrup or elixir can contain the active compound, a sweetener such as sucrose, preservatives like propyl paraben, a colouring agent, and a flavouring agent such as cherry. Sterile compositions for

injection can be formulated according to conventional pharmaceutical practice. For example, dissolution or suspension of the active compound in a vehicle such as water or naturally occurring vegetable oil like sesame, peanut, or cottonseed oil or a synthetic fatty vehicle like ethyl oleate or the like may be
5 desired. Buffers, preservatives, antioxidants, and the like can be incorporated according to accepted pharmaceutical practice.

For injection, intraventricular administration, and other invasive routes of administration, the compounds used must be sterile. Sterility can be accomplished by any method known in the art, for example filtration through
10 sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper able to be pierced by a hypodermic injection needle.

A pharmaceutical formulation ordinarily will be stored in unit or multi-
15 dose containers, for example, in sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10 mL vials are filled with 5 mL of sterile-filtered 1% (w/v) aqueous solution of compound, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized compound
20 using bacteriostatic Water-for-Injection. It can be readily appreciated that other dosage forms and types of preparations can be used, and all are considered to be part of this invention.

Preparation of the Compounds of this Invention

25 Starting materials and reagents used in preparing these compounds are either available from commercial suppliers such as Aldrich Chemical Company (Milwaukee, Wis.), Bachem (Torrance, Calif.), Sigma (St. Louis, Mo.), Strem (Newburyport, Ma.) or are prepared by methods well known to the person of ordinary skill in the art following procedures described in such references as
30 Fieser and Fieser's Reagents for Organic Synthesis, vols 1-17, John Wiley and

Sons, New York, N.Y., 1991; Rodd's Chemistry of Carbon Compounds, vols. 1-5 and supplements, Elsevier Science Publishers, 1989; Organic Reactions, vols. 1-40, John Wiley and Sons, New York, N.Y., 1991; March J; Advanced Organic Chemistry, 4th ed. John Wiley and Sons, New York, N.Y., 1992; and
5 Larock: Comprehensive Organic Transformations, VCH Publishers, 1989. In most instances, amino acids and their esters or amides, and protected amino acids, are widely commercially available; and the preparation of modified amino acids and their amides or esters are extensively described in the chemical and biochemical literature and thus well-known to persons of ordinary skill in
10 the art.

Starting materials, intermediates, and compounds of this invention can be isolated and purified using conventional techniques, including filtration, distillation, crystallization, chromatography, and the like. They can be characterized using conventional methods, including physical constants and
15 spectral data.

All patent and literature references cited throughout the specification are expressly incorporated by reference in their entirety.

EXAMPLES

20 The present invention is further illustrated by the following examples. These examples are offered by way of illustration only and are not intended to limit the invention in any manner.

General methods

Flash chromatography was performed using Scharlau 60 (40-60 μ m
25 mesh) silica gel. Analytical thin layer chromatography was carried out on 0.20 mm pre-coated silica gel plates (ALUGRAM[®] SIL G/UV₂₅₄) and compounds visualized using UV fluorescence, or heating of plates dipped in potassium permanganate in alkaline solution.

Melting points in degrees Celsius (°C) were determined on an Electrothermal[®] melting point apparatus and are uncorrected.

Optical rotations were measured at 20 °C on a Perkin Elmer 341 polarimeter using 10 cm path length cells and are given in units of $10^{-1} \text{deg cm}^2 \text{g}^{-1}$. Samples were prepared in the solvent indicated at the concentration specified (measured in $\text{g}/100 \text{ cm}^3$). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer. The samples were prepared as thin films on sodium chloride discs or as solids in potassium bromide discs. A broad signal indicated by br. The frequencies (ν) as absorption maxima are given in wavenumbers (cm^{-1}).

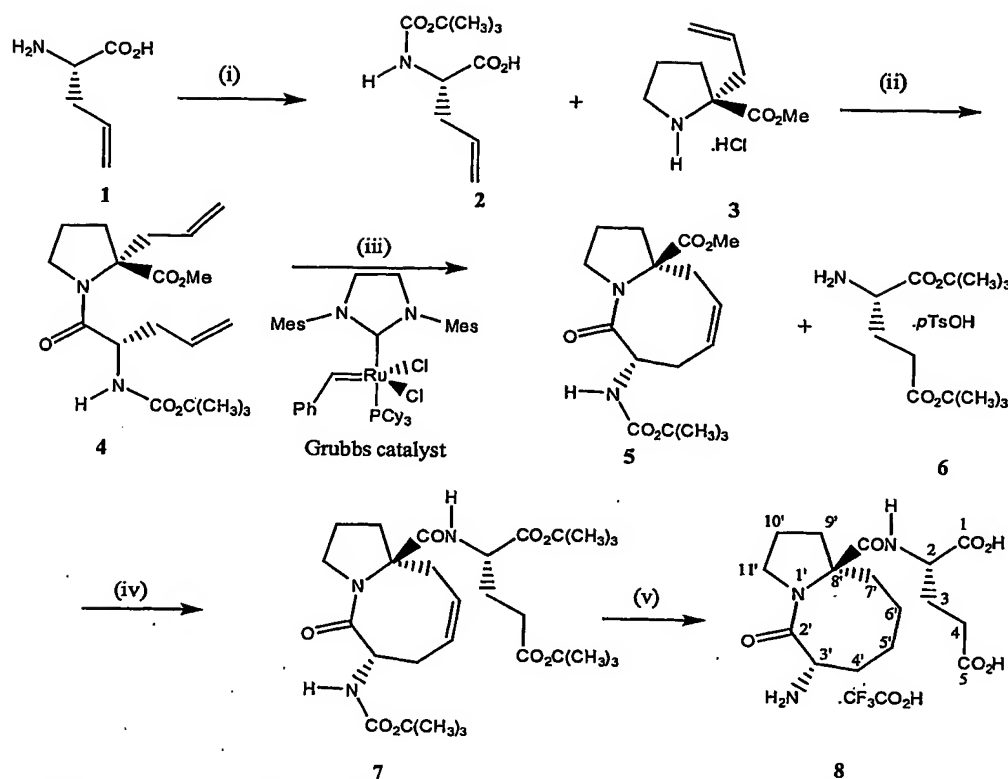
NMR spectra were recorded on a Bruker AVANCE DRX400 (^1H , 400 MHz; ^{13}C , 100 MHz) or a Bruker AVANCE 300 (^1H , 300 MHz; ^{13}C , 75 MHz) spectrometer at ambient temperatures. For ^1H NMR data chemical shifts are described in parts per million downfield from SiMe_4 and are reported consecutively as position (δ_{H}), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, br = broad), coupling constant (J/Hz) and assignment. For ^{13}C NMR data, chemical shifts are described in parts per million relative to CDCl_3 and are reported consecutively as position (δ_{C}), degree of hybridization as determined by DEPT experiments, and assignment. ^1H NMR spectra were referenced internally using SiMe_4 (δ 0.00) or CDCl_3 (δ 7.26). ^{13}C NMR spectra were referenced internally using CDCl_3 (δ 77.0). When two sets of peaks arise in the NMR spectra due to different conformations around the glycine-proline amide bond, the chemical shift for the minor *cis* conformer is marked with an asterisk (*).

Accurate mass measurements were recorded on a VG-70SE mass spectrometer.

Hexane and dichloromethane were distilled prior to use. Methanol was dried using magnesium turnings and iodine, and distilled under nitrogen. Triethylamine was dried over calcium hydride and distilled under nitrogen.

Analytical HPLC was performed on a Waters 600 system using an Alltech Econosphere C18 RP column (150mm x 4.6 mm). A 5 minute flush with water (containing 0.05% trifluoroacetic acid) followed by gradient elution of water (containing 0.05% trifluoroacetic acid) to acetonitrile over 25 mins was used at a flow rate of 1 ml min⁻¹. For semi-preparative purification an Alltech Econosil C18 RP column (250mm x 22mm) was used under isocratic conditions, typically 80% water (containing 0.05% trifluoroacetic acid):20% acetonitrile at a flow rate of 13 ml min⁻¹.

- 10 **Example 1: *Synthesis of (2S, 3'S, 8'S)-2-[[(3'-Amino-1'-aza-2'-oxobicyclo[6.3.0]undecyl)-8'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt***



Scheme 1 **Reagents, conditions and yields:** (i) Boc_2O , NaHCO_3 , H_2O , dioxane, 0°C to RT, overnight (ca. 72%); (ii) DCC, HOBt, Et_3N , CH_2Cl_2 , 0°C to RT, overnight (ca. 23%); (iii) KO^tBu , imidazolium ligand, THF, 2 min then Grubbs catalyst, benzene, 80°C , 35 min then 4, benzene, 45°C , 48 h then DMSO overnight (58%); (iv) 1M aq. NaOH, dioxane, H_2O , RT then 6, BoP-Cl , Et_3N , CH_2Cl_2 , 0°C to RT, 20 h (69%, 2 steps); (v) PtO_2 , H_2 , THF, RT 16 h, then $\text{CF}_3\text{CO}_2\text{H}$, CH_2Cl_2 , RT, 5 h (81%).

5 *N-tert-Butoxy-(S)-allylglycine 2*

A solution of allylglycine 1 (0.1 g, 0.87 mmol) and sodium hydrogen carbonate (0.08 g, 0.96 mmol) in water/dioxane (1:1, v/v, 4 cm^3) was cooled to 0°C , di-*tert*-butyl dicarbonate (0.07 g, 0.32 mmol) was added and the solution stirred for 2 h at 0°C . Di-*tert*-butyl dicarbonate (0.07 g, 0.32 mmol) was then added and the solution stirred for a further 2 h at 0°C , after which time further di-*tert*-butyl dicarbonate (0.1 g, 0.46 mmol) was added and the solution allowed to warm to room temperature and stirred overnight. Dioxane was removed under reduced pressure at 40°C and the resultant residue was dissolved in

water, washed with ether and the aqueous layer acidified with 2M aqueous hydrochloric acid and extracted with ethyl acetate. The combined organic layers were dried (MgSO₄), filtered and the solvent removed *in vacuo* to give crude *carbamate 2* (0.14 g, *ca.* 72%) as a colourless oil. This material was used as such for the subsequent step. Carbamate **2** was shown to be a 73:27 mixture of rotamers: δ_{H} (400 MHz; CDCl₃; Me₄Si) 1.45 [9H, s, C(CH₃)₃], 2.51-2.63 [2H, m, CH₂(allyl)], 4.19* (0.27H, br s, 2-H), 4.41 (0.73H, d, *J* 6.1, 2-H), 5.13-5.20 (2.73H, m, =CH₂, N-H), 5.70-5.80 [1H, m, C(H)=CH₂], 6.26 (0.27H, br s, N-H*) and 9.16 (1H, br s, OH); δ_{C} (100 MHz; CDCl₃) 28.2 [CH₃, C(CH₃)₃], 36.3 [CH₂, CH₂(allyl)], 52.7 (CH, 2-C), 54.2* (CH, 2-C), 80.2 [quat., C(CH₃)₃], 81.6* [quat., C(CH₃)₃], 119.2 (CH₂, =CH₂), 132.1 [CH, C(H)=CH₂], 155.4 (quat., NCO₂), 155.4* (quat., NCO₂), and 176.1 (quat., 1-CO).

N-tert-Butyloxy-(S)-allylglycyl-(S)-allylproline methyl ester 4

N,N'-Dicyclohexylcarbodiimide (0.1 g, 0.49 mmol) was added to a stirred solution of (*S*)-allylproline methyl ester **3** (0.1 g, 0.49 mmol), *N-tert*-butoxy-(*S*)-allylglycine **2** (0.12 g, 0.54 mmol), *N*-hydroxybenzotriazole (0.065 g, 0.486 mmol) and triethylamine (0.07 cm³, 0.486 mmol) in dichloromethane (10 cm³) at 0 °C. The mixture was stirred overnight at room temperature, refrigerated for 2 h and filtered through Celite™ to remove dicyclohexyl urea. The filtrate was washed with saturated aqueous sodium hydrogen carbonate, 2 M aqueous hydrochloric acid, dried (Na₂SO₄), filtered and the solvent removed to yield an oil which was purified by chromatography (SiO₂, 4:1, 3:1, hexane-ethyl acetate) to afford *diene 4* (0.041 g, *ca.* 23%) as a colourless oil. Diene **4** existed exclusively as the *trans* GlyC(O)-NPro conformer: δ_{H} (400 MHz; CDCl₃; Me₄Si) 1.43 [9H, s, C(CH₃)₃], 1.94-2.15 (4H, m, Pro β -H₂, Pro γ -H₂), 2.35 [1H, p, *J* 7.1, allyl-*H_AH_B*(Pro)], 2.50 [1H, p, *J* 7.2, allyl-*H_AH_B*(Pro)], 2.62 [1H, dd, *J* 14.0 and 8.4, allyl-*H_AH_B*(Gly)], 3.18 [1H, dd, *J* 14.1 and 6.5, allyl-

$H_A H_B(\text{Gly})$], 3.65-3.80 (5H, m, OCH_3 and $\text{Pro}\delta\text{-H}_2$), 4.46 (1H, dd, J 15.4 and 6.7, $\text{Gly}\alpha\text{-H}$), 5.08-5.25 (5H, m, 2 x $=\text{CH}_2$ and N-H) and 5.63-5.86 (2H, m, 2 x $\text{C}(\text{H})=\text{CH}_2$); δ_{C} (100 MHz; CDCl_3) 23.6 (CH_2 , $\text{Pro}\gamma\text{-C}$), 28.2 [CH_3 , $\text{C}(\text{CH}_3)_3$], 35.0 (CH_2 , $\text{Pro}\beta\text{-C}$), 36.3 [CH_2 , $\text{CH}_2(\text{allyl})\text{Gly}$], 37.7 [CH_2 , $\text{CH}_2(\text{allyl})\text{Pro}$], 48.5
 5 (CH_2 , $\text{Pro}\delta\text{-C}$), 51.6 (CH , $\text{Gly}\alpha\text{-C}$), 52.2 (CH_3 , OCH_3), 68.5 (quat., $\text{Pro}\alpha\text{-C}$), 79.6 [quat., $\text{C}(\text{CH}_3)_3$], 118.5 (CH_2 , $=\text{CH}_2$), 119.1 (CH_2 , $=\text{CH}_2$), 132.7 [CH , $\text{C}(\text{H})=\text{CH}_2$], 133.0 [CH , $\text{C}(\text{H})=\text{CH}_2$], 155.4 (quat., NCO_2), 170.2 (quat., Gly-CO) and 173.7 (quat., Pro-CO).

10 *(3S, 8S)-1-Aza-3-(tert-butyloxycarbonylamino)-8-methoxycarbonyl-2-oxobicyclo[6.3.0]undec-5-ene 5*

A solution of freshly sublimed potassium tert-butoxide (0.0018 g, 0.0157 mmol) in dry tetrahydrofuran (1 cm^3) was added to a stirred suspension of 1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydro-imidazolium tetrafluoroborate
 15 (0.07 g, 0.0177 mmol) in dry tetrahydrofuran (2 cm^3) under an atmosphere of nitrogen. Dry tetrahydrofuran (1 cm^3) was used to rinse the remaining potassium tert-butoxide from the reaction flask. The resultant suspension was stirred for 2 min then a solution of
 bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs's catalyst)
 20 (0.010 g, 0.011 mmol) in dry benzene (10 cm^3) was added and the purple solution heated at 80 °C for 35 min. The dark brown solution was cooled to room temperature and a solution of diene 4 (0.041 g, 0.112 mmol) in dry benzene (40 cm^3) added and the mixture heated at 45 °C for 48 h. The brown solution was cooled to room temperature, dimethyl sulphoxide (0.043 g, 0.56
 25 mmol) was added and the mixture stirred overnight. The solvent was removed in vacuo and the residue purified by chromatography (SiO_2 , 2:1, 1:1, hexane-ethyl acetate) to give alkene 5 (0.022 g, 58%) as a greenish oil. Alkene 5 existed exclusively as the trans $\text{C}(\text{O})\text{-NPro}$ conformer: $[\alpha]_{\text{D}} -86.3$ (c 0.183 in CH_2Cl_2) [lit.², -87.4 (c 0.35 in CHCl_3); δ_{H} (400 MHz; CDCl_3 ; Me_4Si) 1.44 [9H,

s, C(CH₃)₃], 1.66-1.86 (2H, m, 10-H₂), 1.96-2.11 (2H, m, 9-H_AH_B and 4-H_AH_B), 2.46 (1H, dd, J 12.3 and 6.1, 9-H_AH_B), 2.66 (1H, dd, J 14.8 and 8.2, 7-H_AH_B), 2.80-2.90 (1H, m, 4-H_AH_B), 3.04 (1H, dd, J 15.1 and 8.6, 7-H_AH_B), 3.40 (1H, ddd, J 11.6, 11.1 and 7.4, 11-H_AH_B), 3.76-3.83 (1H, m, 11-H_AH_B), 3.78 (3H, s, OCH₃), 4.97-5.04 (1H, m, 3-H), 5.52-5.63 (2H, m, 6-H and N-H) and 5.74-5.79 (1H, m, 5-H); δ_C (100 MHz; CDCl₃) 20.6 (CH₂, 10-C), 28.2 [CH₃, C(CH₃)₃], 35.0 (CH₂, 7-C), 35.4 (CH₂, 4-C), 38.4 (CH₂, 9-C), 48.4 (CH₂, 11-C), 50.8 (CH, 3-C), 53.0 (CH₃, OCH₃), 69.7 (quat., 8-C), 79.4 [quat., C(CH₃)₃], 122.8 (CH, 5-C), 132.2 (CH, 6-C), 154.8 (quat., NCO₂), 171.5 (quat., 2-C) and 173.7 (quat., 8-CO).

(2S, 3'S, 8'S)-Di-tert-butyl 2-{[(1'-aza-3'-(tert-butyloxycarbonylamino)--2'-oxobicyclo[6.3.0]undec-5'-ene)-8'-carbonyl]amino}-1,5-pentadioate 7

To a solution of alkene 5 (0.022 g, 0.065) in dioxane (0.7 cm³) was added 1 M aqueous sodium hydroxide (0.32 cm³, 0.32 mmol) and the opaque solution stirred at room temperature for 17 h. Water was added and the mixture washed with dichloromethane. The aqueous layer was acidified with citric acid and the product extracted with dichloromethane. The organic layers were pooled, dried (MgSO₄) and the solvent removed to afford an oil (0.020 g). To a solution of this oil in dichloromethane (4 cm³) was added L-glutamic acid di-tert-butyl ester p-toluenesulphonate 6 (0.025 g, 0.085 mmol) was added and the solution cooled to 0 °C. Triethylamine (0.017 g, 0.17 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.021 g, 0.085 mmol) were added and the solution stirred for 20 h. The reaction mixture was washed with 2M aqueous hydrochloric acid, saturated aqueous sodium hydrogen carbonate, dried (Na₂SO₄), filtered and the solvent removed to yield an oil (0.041 g) which was purified by chromatography (SiO₂, 1:1, 2:3, hexane-ethyl acetate) to give amide 7 (0.025 g, 69% in 2 steps) as a colorless oil. Amide 7 existed exclusively as the cis C(O)-NPro conformer: $[\alpha]_D$ -49.6 (c 0.25 in CH₂Cl₂); δ_H (400 MHz;

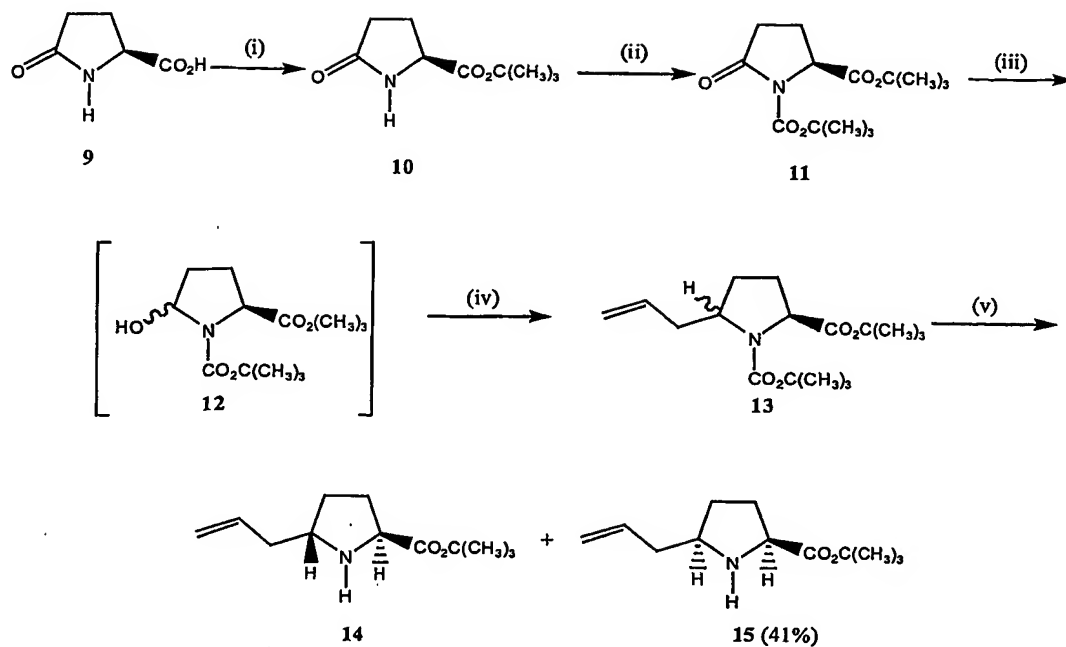
CDCl₃; Me₄Si) 1.40-1.44 [27H, m, 3 x C(CH₃)₃], 1.55-1.66 (1H, m), 1.78 (1H, p, J 6.4), 1.89 (1H, td, J 13.2 and 6.1), 2.05-2.39 (5H, m), 2.65-2.75 (2H, m, 4'-H₂), 2.80 (1H, dd, J 15.0 and 7.9, 7'-H_AH_B), 2.92 (1H, dd, J 14.9 and 8.9, 7'-H_AH_B), 3.55 (1H, ddd, J 11.8, 11.8 and 7.0, 11'-H_AH_B), 3.65-3.70 (1H, m, 11'-H_AH_B), 4.40-4.46 (1H, m, 3'-H), 4.88-4.97 (2H, m, 6'-H and 2-H), 5.67-5.79 (2H, m, 5'-H and N-H) and 8.36 (1H, d, J 8.4, N-H); δ_C (100 MHz; CDCl₃) 20.4 (CH₂, 10'-C), 25.4 (CH₂, 3-C), 27.9 [CH₃, C(CH₃)₃], 28.0 [CH₃, C(CH₃)₃], 28.2 [CH₃, C(CH₃)₃], 31.5 (CH₂, 4-C), 32.2 (CH₂, 4'-C), 34.6 (CH₂, 7'-C), 37.4 (CH₂, 9'-C), 49.3 (CH₂, 11'-C), 52.1 (CH, 3'-C), 52.8 (2-C), 71.8 (quat., 8'-C), 80.4 [quat., C(CH₃)₃], 81.1 [quat., C(CH₃)₃], 125.0 (CH, 5'-C), 130.2 (CH, 6'-C), 156.3 (quat., NCO₂), 170.5 (quat., 2'-C), 171.9 (quat., 8'-CO), 171.2 (quat., 1-C) and 173.5 (quat., 5-C); m/z (EI+) 565.3365 (M⁺. C₂₉H₄₇N₃O₈ requires 565.3363).

15 (2*S*, 3'*S*, 8'*S*) 2-[[[(1'-Aza-3'-amino-2'-oxobicyclo[6.3.0]undecyl)-8'-carbonyl]amino]-1,5-pentandioic acid trifluoroacetate salt **8**

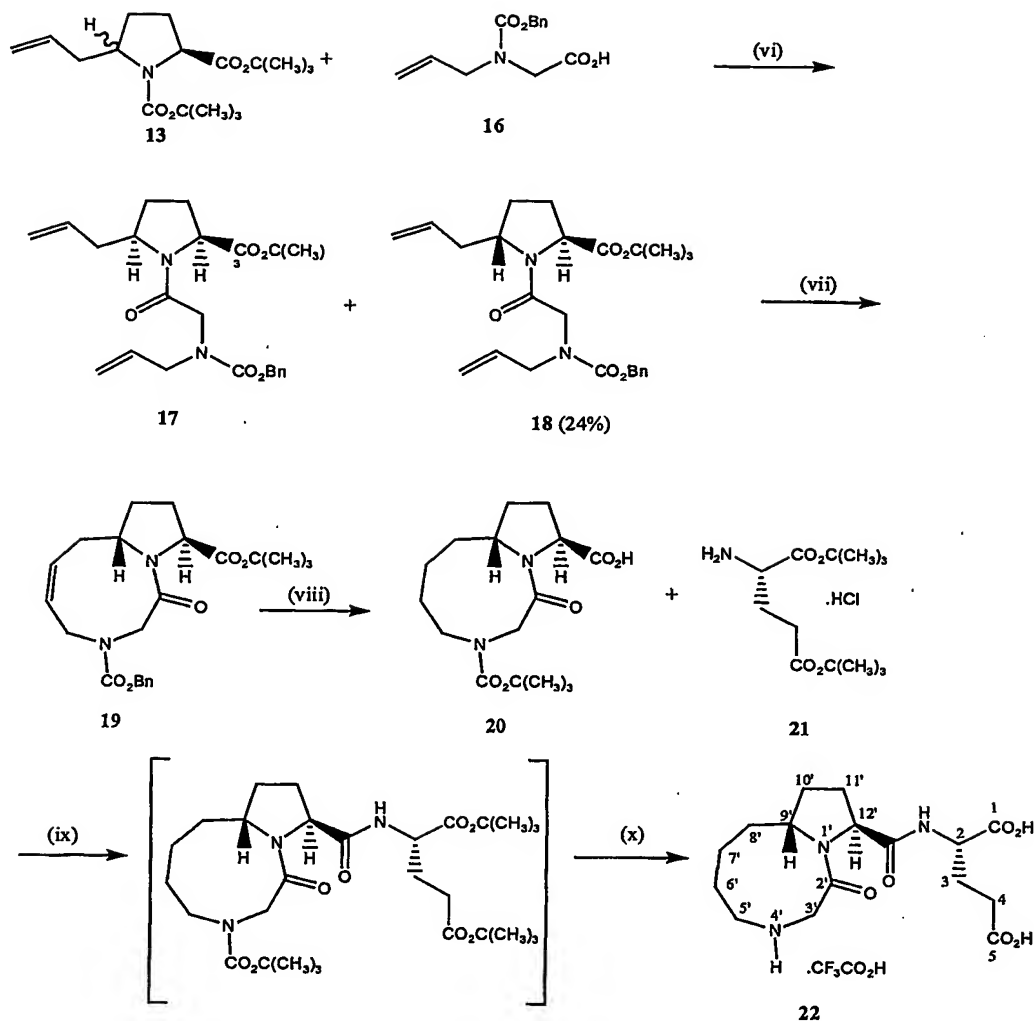
PtO₂ (0.001 g, 0.004 mmol) was added to a stirred solution of amide 7 (0.025 g, 0.044 mmol) in tetrahydrofuran (4 cm³) under a nitrogen atmosphere. The mixture was hydrogenated (1 atm. of hydrogen) for 17 h, filtered through
 20 CeliteTM, and the solvent removed in vacuo. The residue was dissolved in dichloromethane (3 cm³), trifluoroacetic acid (1 cm³) added and the solution stirred for 4 h at room temperature. Removal of the volatiles in vacuo and analysis by HPLC and NMR showed the reaction to be incomplete. The residue was therefore redissolved in dichloromethane-trifluoroacetic acid (3:1, 4 cm³)
 25 and the solution stirred at room temperature for 2.5 h. The volatiles were removed in vacuo, and the residue purified by RP HPLC [20% acetonitrile:80% water (containing 0.05% trifluoroacetic acid)] to give an oil which was triturated from ether/toluene to give **8** (0.0167 g, 81%, in 2 steps) as a hygroscopic white solid. Macrocyclic **8** existed exclusively as the *cis* C(O)-NPro conformer: mp

75-85 °C; $[\alpha]_D$ -4.4 (c 0.16 in MeOH); δ_H (400 MHz; D₂O) 1.30-1.39 (1H, m, 5'-H_AH_B), 1.57-1.65 (1H, m, 6'-H_AH_B), 1.79-1.91 (4H, m, 5'-H_AH_B, 6'-H_AH_B, 4'-H_AH_B and 10'-H_AH_B), 1.98-2.13 (4H, m, 4'-H_AH_B, 10'-H_AH_B, 7'-H_AH_B and 3'-H_AH_B), 2.23-2.37 (4H, m, 9'-H₂, 3-H_AH_B and 7'-H_AH_B), 2.53 (2H, t, J 7.5, 4-H₂), 3.68 (1H, dt, J 14.2 and 7.4, 11'-H_AH_B), 3.79 (1H, dt, J 14.0 and 7.5, 11'-H_AH_B), 4.20 (1H, dd, J 11.2 and 6.3, 3-H) and 4.53 (1H, dd, J 10.2 and 4.9, 2-H); δ_C (100 MHz; D₂O) 20.4 (CH₂, 10'-C), 21.2 (CH₂, 6'-C), 22.1 (CH₂, 5'-C), 24.8 (CH₂, 3-C), 30.3 (CH₂, 4-C), 31.1 (CH₂, 4'-C), 36.4 (CH₂, 7'-C), 40.9 (CH₂, 9'-C), 49.1 (CH₂, 11'-C), 51.2 (CH, 3'-C), 52.4 (CH, 2-C), 70.9 (quat., 8'-C), 116.1 (quat., q, J 291, CF₃), 162.7 (quat., q, J 36.0, CF₃CO₂H), 169.4 (quat., 2'-C), 174.6 (quat., 8'-CO), 175.3 (quat., 1-C) and 177.0 (quat., 5-C); m/z (FAB+) 356.1830 [MH(free base)⁺. C₁₆H₁₆N₃O₆ requires 356.1822].

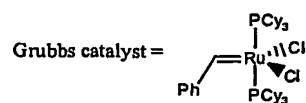
Example 2: Synthesis of (2*S*, 9'*S*, 12'*S*)-2-[[*(1',4'*-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt



Scheme 1 Reagents, conditions and yields: (i) 70% (aq) HClO_4 , *tert*-BuOAc, RT, 24 h (56%); (ii) DMAP, Boc_2O , CH_3CN , RT, 19 h (95%); (iii) LiEt_3BH , THF, -78°C , 1 h, then 30% H_2O_2 , 0°C , 0.5 h; (iv) Me_3SiOTf , allyltributyltin, CH_2Cl_2 , -78°C , 2 h (70%, 2 steps); (v) 4M HCl, dioxane, 0°C 1 h, then RT, 40 min (72%).



Scheme 2 Reagents, conditions and yields: (vi) 4M HCl, dioxane, 0 °C 1h, then RT, 40 min, then 8, EDCI, CH_2Cl_2 , Et_3N , 0 °C to RT, 16 h (64% over 2 steps); (vii) 9, Grubbs' catalyst, CH_2Cl_2 , reflux, 48 h, then DMSO RT, 24 h (42%); (viii) PtO_2 , H_2 , THF, RT, 16 h then 30% HBr/HOAc, RT, 2 h, then NaHCO_3 , H_2O , dioxane, Boc_2O , RT, 3 d (58%, 3 steps); (ix) BoP-Cl , Et_3N , 0 °C to RT, 17 h; (x) CH_2Cl_2 , $\text{CF}_3\text{CO}_2\text{H}$, RT, 6 h (55% 2 steps).



(S)-tert-Butyl pyroglutamate 10

Perchloric acid (70% aqueous, 0.521 cm³, 6.05 mmol) was added dropwise to a stirred suspension of (*S*)-pyroglutamic acid **9** (1 g, 7.75 mmol) in *tert*-butyl acetate (12 cm³, 85.5 mmol). The mixture was stirred for 24 h then
5 sodium carbonate (0.80 g, 7.60 mmol) was added cautiously, ether was added and the organic phase washed with saturated sodium hydrogen carbonate solution and brine. The aqueous phase was extracted with ethyl acetate, dried (MgSO₄), filtered and the solvent removed to yield an oil to which was added carbon tetrachloride and the solvent evaporated *in vacuo* to remove traces of
10 acetic acid to give *ester 10* (0.81 g, 56%) as a white solid: δ_{H} (300 MHz; CDCl₃; Me₄Si) 1.48 [9H, s, C(CH₃)], 2.33-2.45 (4H, m, 3-H₂, 4-H₂), 4.12-4.16 (1H, m, 5-H) and 6.16 (1H, br s, N-H); δ_{C} (75 MHz; CDCl₃) 24.7 (CH₂, 4-C), 27.8 [CH₃, C(CH₃)], 29.2 (CH₂, 3-C), 55.9 (CH, 5-C), 82.3 [quat., C(CH₃)], 170.9 (quat., 5-CO) and 177.7 (quat., 2-C).

15

(S)-tert-Butyl N-tert-butoxycarbonylpyroglutamate 11

To a solution of *ester 10* (0.85 g, 4.58 mmol) and dimethylaminopyridine (0.056 g, 0.458 mmol) in acetonitrile (40 cm³) was added di-*tert*-butyl dicarbonate (1.12 g, 5.50 mmol). The resultant solution was
20 stirred at room temperature for 19 h, concentrated *in vacuo* and purified by chromatography (SiO₂, 4:1, 3:1 hexane-ethyl acetate; gradient elution) to give *carbamate 11* (1.24 g, 95%) as a white solid: mp 53-55 °C (lit. 54-56 °C); δ_{H} (300 MHz; CDCl₃; Me₄Si) 1.48 [9H, s, C(CH₃)₃], 1.50 [9H, s, C(CH₃)₃], 1.93-2.03 (1H, m), 2.16-2.35 (1H, m), 2.40-2.66 (2H, m) and 4.47 (1H, dd, *J* 9.4 2.7, 5-H); δ_{C} (75 MHz; CDCl₃) 21.6 (CH₂, 4-C), 27.8 [CH₃, C(CH₃)₃], 31.0 (CH₂, 3-C), 59.5 (CH, 5-C), 82.2 [quat., C(CH₃)], 83.2 [quat., C(CH₃)₃], 149.2 (quat., NCO₂), 170.3 (quat., 5-CO) and 173.4 (quat., 2-C).

25

N-tert-Butoxycarbonyl-5-allyl-L-proline tert-butyl ester 13

To a stirred solution of carbamate **11** (1.14 g, 3.98 mmol) in dry tetrahydrofuran (40 cm³) was added a 1 mol L⁻¹ tetrahydrofuran solution of lithium triethylborohydride (4.78 cm³, 4.78 mmol) at -78 °C under at
 5 atmosphere of nitrogen. The solution was stirred for 1 h, saturated aqueous sodium hydrogen carbonate (10 cm³) added and the cooling bath removed. The temperature was allowed to reach 0 °C then 30% aqueous hydrogen peroxide (30 drops) was added and stirring was continued at 0 °C for 30 min. The aqueous layer was extracted with ether and the combined organic extracts were
 10 washed with brine, dried (MgSO₄), filtered and the solvent removed to give *alcohol 12* (1.17 g) which contained ~8% of **11** as determined by ¹H NMR. The crude *alcohol 12* was dissolved in dry dichloromethane (20 cm³) and added dropwise to a stirred solution of trimethylsilyl triflate (1.44 cm³, 7.96 mmol) and allyltributylstannane (2.46 cm³, 7.96 mmol) in dry dichloromethane (20
 15 cm³) at -78 °C under nitrogen. The solution was stirred at -78 °C for 2 h, saturated aqueous sodium hydrogen carbonate (15 cm³) was added and the reaction warmed to room temperature. The aqueous layer was extracted with dichloromethane and the pooled organic extracts dried (MgSO₄) and filtered. Removal of the solvent *in vacuo* gave an oil (3.855 g) which was purified by
 20 chromatography (SiO₂, 8:1, 7:1, hexane-ethyl acetate; gradient elution) to afford an oil (1.047 g) contaminated with tributylstannane residues. Further chromatography (SiO₂, 12:1, 10:1, 8:1, hexanes-ethyl acetate; gradient elution) gave *alkene 13* (0.872 g, 70%, in 2 steps) as a colourless oil. *Alkene 13* was shown to be a 57:43 mixture of C(2)/C(5) *cis:trans* isomers: δ_H (300 MHz; CDCl₃; Me₄Si) 1.41-1.45 [18H, m, 2 x C(CH₃)₃], 1.67-2.24 [5H, m, Pro β -H₂, Pro γ -H₂ and CH_ACH_B(allyl)], 2.39-2.71 [1H, m, CH_ACH_B(allyl)], 3.79-4.18 (2H, m, Pro α -H and Pro δ -H), 4.99-5.08 (2H, m, =CH₂) and 5.67-5.85 [1H, m, C(H)=CH₂]; δ_C (75 MHz; CDCl₃) 26.6 (CH₂, Pro γ -C), 27.4 (CH₂, Pro γ -C), 27.8 (CH₂, Pro γ -C), 27.86 [CH₃, C(CH₃)₃], 27.92 [CH₃, C(CH₃)₃], 28.25 [CH₃, C(CH₃)₃], 28.31 [CH₃, C(CH₃)₃], 28.4 (CH₂, Pro β -C), 29.4 (CH₂, Pro β -C), 30.2

(CH₂, Proβ-C), 38.1 [CH₂, CH₂(allyl)], 38.5 [CH₂, CH₂(allyl)], 38.98 [CH₂, CH₂(allyl)], 39.1 [CH₂, CH₂(allyl)], 57.4 (CH, Proδ-C), 58.0 (CH, Proδ-C), 60.6 (CH, Proα-C), 60.9 (CH, Proα-C), 79.5 [quat., C(CH₃)₃], 79.6 [quat., C(CH₃)₃], 80.7 [quat., C(CH₃)₃], 80.74 [quat., C(CH₃)₃], 116.9 (CH₂, =CH₂), 116.99 (CH₂, =CH₂), 117.0 (CH₂, =CH₂), 135.12 (CH, C(H)=CH₂), 135.2 (CH, C(H)=CH₂), 135.5 (CH, C(H)=CH₂), 153.7 (quat., NCO₂), 153.8 (quat., NCO₂), 154.3 (quat., NCO₂), 172.1 (quat., Proα-CO), 172.2 (quat., Proα-CO) and 172.3 (quat., Proα-CO); *m/z* (EI⁺) 312.2171 (M⁺. C₁₇H₃₀NO₄ requires 312.2175).

10 *(1S, 2S)-5-Allylproline tert-butyl ester (trans) 14 and (1S, 2R) 5-allylproline tert-butyl ester (cis) 15*

To alkene **13** (0.24 g, 0.77 mmol) was added a 4 mol L⁻¹ solution of hydrogen chloride in dioxane (4 cm³) at 0 °C. The solution was stirred for 1 h at 0 °C then room temperature for 40 min at which time reaction was complete (by tlc). The mixture was cooled to 0 °C, neutralized with a saturated solution of aqueous sodium hydrogen carbonate and the product extracted with dichloromethane. Removal of the solvent *in vacuo* yielded an oil (0.142 g) which was purified by chromatography (SiO₂, 2:1, 5:4, 1:1, hexanes-ethyl acetate) to give (i) *amine 14 (trans)* (0.051 g, 31%) as a colourless oil. This compound showed no NOE between the Proα-H atom at δ3.70 and the Proδ-H atom at δ3.26 : [α]_D -31 (c 0.47 in CH₂Cl₂); δ_H (300 MHz; CDCl₃; Me₄Si) 1.35-1.41 (1H, m, Proγ-H_AH_B), 1.43 [9H, m, C(CH₃)₃], 1.72-1.90 (2H, Proγ-H_AH_B and Proβ H_AH_B), 2.11-2.20 [3H, m, Proβ H_AH_B and CH₂(allyl)], 2.47 (1H, s, N-H), 3.26 (1H, p, *J* 6.5, Proδ-H), 3.70 (1H, dd, *J* 8.5 and 5.9, Proα-H), 4.98-5.10 (2H, m, =CH₂) and 5.73-5.84 [1H, m, C(H)=CH₂]; δ_C (75 MHz; CDCl₃) 27.9 [CH₃, C(CH₃)₃], 29.5 (CH₂, Proβ-C), 30.7 (CH₂, Proγ-C), 40.8 [CH₂, CH₂(allyl)], 57.6 (CH, Proδ-C), 59.7 (CH, Proα-C), 80.7 [quat., C(CH₃)₃], 116.1 (CH₂, =CH₂), 136.0 [CH, C(H)=CH₂], and 174.9 (quat., Proα-CO); (ii) *amine 15 (cis)* (0.066 g, 41%) as a colourless oil. This compound

showed an NOE between the Pro α -H atom at δ 3.57-3.62 and the Pro δ -H atom at δ 3.03-3.12: $[\alpha]_D -22.1$ (c 0.66 in CH₂Cl₂); δ_H (300 MHz; CDCl₃; Me₄Si) 1.24-1.30 (1H, m, Pro γ -H_AH_B), 1.42 [9H, m, C(CH₃)₃], 1.78-1.87 (2H, Pro γ -H_AH_B and Pro β -H_AH_B), 1.98-2.07 (1H, m, Pro β -H_AH_B), 2.18-2.31 [3H, m, CH₂(allyl) and N-H], 3.03-3.12 (1H, m, Pro δ -H), 3.57-3.62 (1H, m, Pro α -H), 4.99-5.1 (2H, m, =CH₂) and 5.72-5.86 [1H, m, C(H)=CH₂]; δ_C (75 MHz; CDCl₃) 27.9 [CH₃, C(CH₃)₃], 30.4 (CH₂, Pro β -C), 31.1 (CH₂, Pro γ -C), 39.9 [CH₂, CH₂(allyl)], 59.1 (CH, Pro δ -C), 60.5 (CH, Pro α -C), 80.8 [quat., C(CH₃)], 116.5 (CH₂, =CH₂), 135.5 [CH, C(H)=CH₂], and 174.3 (quat., Pro α -CO); m/z (CI⁺) 212.1647 (MH⁺. C₁₂H₂₂NO₂ requires 212.1651).

(1*S*, 2*S*)-*N*-Allyl-*N*-benzyloxycarbonylglycine-5-allylproline *tert*-butyl ester (*trans*) **18** and (1*S*, 2*R*) *N*-allyl-*N*-benzyloxycarbonylglycine-5-allylproline *tert*-butyl ester (*cis*) **17**

To alkene **13** (0.87 g, 0.77 mmol) was added a 4M solution of hydrogen chloride in dioxane (4 cm³) at 0 °C. The solution was stirred for 1 h at 0 °C then room temperature for 35 min after which time reaction was complete (by tlc). The mixture was cooled to 0 °C, neutralized with a saturated solution of aqueous sodium hydrogen carbonate and the product extracted with dichloromethane. Removal of the solvent *in vacuo* yielded an oil (0.568 g) that was dissolved in dichloromethane (30 cm³). To this was added triethylamine (0.54 cm³, 4.0 mmol), *N*-allyl-*N*-benzyloxycarbonylglycine **16** (1.0 g, 4.0 mmol) and 1-(3-dimethylaminopropyl)3-ethylcarbodiimide hydrochloride (EDCI) (0.77 g, 4.0 mmol) at 0 °C and the solution stirred for 16 h. The mixture was washed with saturated aqueous sodium hydrogen carbonate, 2 M aqueous hydrochloric acid, dried (MgSO₄), filtered and the solvent removed to yield an oil (1.7 g) which was purified by chromatography (SiO₂, 5:1, 4:1, 3:1, 2:1, hexane-ethyl acetate,) to afford (i) diene **18** (*trans*) (0.29 g, 24%) as a colourless oil. Diene **18** was shown to be a 1:1 *trans*:*cis* mixture of GlyC(O)-

NPro conformers. In addition, restricted rotation about the GlyN-CO carbamate bond was also observed resulting in a 1:1 mixture of conformers: $[\alpha]_D -56.1$ (c 0.77 in CH_2Cl_2); δ_H (400 MHz; CDCl_3 ; Me_4Si) 1.43 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.46 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.48 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.74-2.71 [6H, m, $\text{Pro}\beta\text{-H}_2$, $\text{Pro}\gamma\text{-H}_2$ and $\text{CH}_2(\text{allyl})$], 3.54 (0.5H, d, J 16.5, $\text{Gly}\alpha\text{-H}_A\text{H}_B$), 3.58 (0.5H, d, J 16.5, $\text{Gly}\alpha\text{-H}_A\text{H}_B$), 3.84-4.40 [5H, m, $\text{Gly}\alpha\text{-H}$, $\text{CH}_2(\text{allyl})$, $\text{Pro}\alpha\text{-H}$ and $\text{Pro}\delta\text{-H}$], 4.98-5.25 (6H, m, OCH_2Ph and 2 x $=\text{CH}_2$), 5.56-5.88 [2H, m, 2 x $\text{C}(\text{H})=\text{CH}_2$] and 7.30-7.37 (5H, m, Ph); δ_C (100 MHz; CDCl_3) 25.8 (CH_2 , $\text{Pro}\beta\text{-C}$ or $\text{Pro}\gamma\text{-C}$), 25.9 (CH_2 , $\text{Pro}\beta\text{-C}$ or $\text{Pro}\gamma\text{-C}$), 26.16 (CH_2 , $\text{Pro}\beta\text{-C}$ or $\text{Pro}\gamma\text{-C}$), 26.2 (CH_2 , $\text{Pro}\beta\text{-C}$ or $\text{Pro}\gamma\text{-C}$), 27.8 [CH_3 , $\text{C}(\text{CH}_3)_3$], 28.3 (CH_2 , $\text{Pro}\beta\text{-C}$ or $\text{Pro}\gamma\text{-C}$), 29.3 (CH_2 , $\text{Pro}\beta\text{-C}$ or $\text{Pro}\gamma\text{-C}$), 29.4 (CH_2 , $\text{Pro}\beta\text{-C}$ or $\text{Pro}\gamma\text{-C}$), 36.9 [CH_2 , $\text{CH}_2(\text{allyl})$], 37.0 [CH_2 , $\text{CH}_2(\text{allyl})$], 39.0 [CH_2 , $\text{CH}_2(\text{allyl})$], 39.3 [CH_2 , $\text{CH}_2(\text{allyl})$], 47.4 (CH_2 , $\text{Gly}\alpha\text{-C}$), 47.6 (CH_2 , $\text{Gly}\alpha\text{-C}$), 47.8 (CH_2 , $\text{Gly}\alpha\text{-C}$), 48.1 (CH_2 , $\text{Gly}\alpha\text{-C}$), 50.14 [CH_2 , $\text{NCH}_2(\text{allyl})$], 50.18 [CH_2 , $\text{NCH}_2(\text{allyl})$], 50.7 [CH_2 , $\text{NCH}_2(\text{allyl})$], 50.8 [CH_2 , $\text{NCH}_2(\text{allyl})$], 56.9 (CH, $\text{Pro}\delta\text{-C}$), 57.1 (CH, $\text{Pro}\delta\text{-C}$), 58.2 (CH, $\text{Pro}\delta\text{-C}$), 58.3, (CH, $\text{Pro}\delta\text{-C}$), 60.0 (CH, $\text{Pro}\alpha\text{-C}$), 60.1 (CH, $\text{Pro}\alpha\text{-C}$), 60.2 (CH, $\text{Pro}\alpha\text{-C}$), 60.3 (CH, $\text{Pro}\alpha\text{-C}$), 67.3 (CH_2 , OCH_2Ph), 67.4 (CH_2 , OCH_2Ph), 81.1 [quat., $\text{C}(\text{CH}_3)_3$], 82.3 [quat., $\text{C}(\text{CH}_3)_3$], 116.9 (CH_2 , $=\text{CH}_2$), 117.1 (CH_2 , $=\text{CH}_2$), 117.2 (CH_2 , $=\text{CH}_2$), 117.6 (CH_2 , $=\text{CH}_2$), 117.7 (CH_2 , $=\text{CH}_2$), 118.1 (CH_2 , $=\text{CH}_2$), 118.2, (CH_2 , $=\text{CH}_2$), 127.6 (CH, Ph), 127.8 (CH, Ph), 127.9 (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 133.3 [CH, $\text{C}(\text{H})=\text{CH}_2$], 133.4 [CH, $\text{C}(\text{H})=\text{CH}_2$], 133.6 [CH, $\text{C}(\text{H})=\text{CH}_2$], 133.8 [CH, $\text{C}(\text{H})=\text{CH}_2$], 134.9 [CH, $\text{C}(\text{H})=\text{CH}_2$], 135.0 [CH, $\text{C}(\text{H})=\text{CH}_2$], 136.5 (quat., Ph), 156.0 (quat., NCO_2), 156.37 (quat., NCO_2), 156.42, (quat., NCO_2), 167.1 (quat., Gly-CO), 167.3 (quat., Gly-CO), 167.4 (quat., Gly-CO), 167.6 (quat., Gly-CO) and 170.9 (quat., $\text{Pro}\alpha\text{-CO}$), 171.0 (quat., $\text{Pro}\alpha\text{-CO}$), 171.1 (quat., $\text{Pro}\alpha\text{-CO}$); m/z (EI⁺) 442.2455 (M^+ . $\text{C}_{25}\text{H}_{34}\text{N}_2\text{O}_5$ requires 442.2468); (ii) a mixture of 17 and 18 (0.044 g, 4%); (iii) diene 17 (*cis*) (0.424 g, 36%) as a colourless oil. Diene 17 was shown to be a 1:1 *trans:cis* mixture of GlyC(O)-NPro

conformers. In addition, restricted rotation about the GlyN-CO carbamate bond was also observed resulting in a 1:1 mixture of conformers: $[\alpha]_D -39.2$ (c 0.57 in CH_2Cl_2); δ_H (400 MHz; CDCl_3 ; Me_4Si) 1.43 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.45 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.47 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.49 [9H, s, $\text{C}(\text{CH}_3)_3$], 1.69-2.49 [5.75H, m, Pro β -H₂, Pro γ -H₂ and $\text{CH}_2(\text{allyl})$], 2.78-2.84 [0.25H, m, $\text{CH}_2(\text{allyl})$], 3.61-4.41 [6H, m, Gly α -H₂, $\text{CH}_2(\text{allyl})$, Pro α -H and Pro δ -H], 5.02-5.12 (6H, m, OCH_2Ph and 2 x =CH₂), 5.67-5.87 [2H, m, 2 x C(H)=CH₂] and 7.29-7.34 (5H, m, Ph); δ_C (100 MHz; CDCl_3) 26.5 (CH_2 , Pro β -C), 27.7 [CH_3 , $\text{C}(\text{CH}_3)_3$], 27.8 [CH_3 , $\text{C}(\text{CH}_3)_3$], 27.9 [CH_3 , $\text{C}(\text{CH}_3)_3$], 29.4 (CH_2 , Pro β -C or Pro γ -C), 29.6 (CH_2 , Pro β -C or Pro γ -C), 29.7 (CH_2 , Pro β -C or Pro γ -C), 37.65 [CH_2 , $\text{CH}_2(\text{allyl})$], 37.7 [CH_2 , $\text{CH}_2(\text{allyl})$], 38.9 [CH_2 , $\text{CH}_2(\text{allyl})$], 47.2 (CH_2 , Gly α -C), 47.6 (CH_2 , Gly α -C), 47.7 (CH_2 , Gly α -C), 48.0 (CH_2 , Gly α -C), 50.2 [CH_2 , $\text{NCH}_2(\text{allyl})$], 50.3 [CH_2 , $\text{NCH}_2(\text{allyl})$], 50.8 [CH_2 , $\text{NCH}_2(\text{allyl})$], 50.9 [CH_2 , $\text{NCH}_2(\text{allyl})$], 57.8 (CH, Pro δ -C), 58.5 (CH, Pro δ -C), 60.2, 60.4 (CH, Pro α -C), 60.5 (CH, Pro α -C), 67.3 (CH_2 , OCH_2Ph), 67.4 (CH_2 , OCH_2Ph), 81.0 [quat., $\text{C}(\text{CH}_3)_3$], 82.2 [quat., $\text{C}(\text{CH}_3)_3$], 116.9 (CH_2 , =CH₂), 117.0 (CH_2 , =CH₂), 117.5 (CH_2 , =CH₂), 118.0 (CH_2 , =CH₂), 127.6 (CH, Ph), 127.8 (CH, Ph), 127.9 (CH, Ph), 128.0 (CH, Ph), 128.3 (CH, Ph), 133.3 [CH, C(H)=CH₂], 133.4 [CH, C(H)=CH₂], 133.5 [CH, C(H)=CH₂], 133.6 [CH, C(H)=CH₂], 134.1 [CH, C(H)=CH₂], 134.3 [CH, C(H)=CH₂], 134.8 [CH, C(H)=CH₂], 134.9 [CH, C(H)=CH₂], 136.5 (quat., Ph), 155.8 (quat., NCO_2), 155.9 (quat., NCO_2), 156.38 (quat., NCO_2), 156.44 (quat., NCO_2), 166.95 (quat., Gly-CO), 167.01 (quat., Gly-CO), 167.4 (quat., Gly-CO), 167.5 (quat., Gly-CO), 171.0 (quat., Pro α -CO), 172.2 (quat., Pro α -CO) and 171.3 (quat., Pro α -CO); m/z (EI+) 442.2462 (M^+ . $\text{C}_{25}\text{H}_{34}\text{N}_2\text{O}_5$ requires 442.2468).

(6Z, 9S, 12S) N-Benzoyloxycarbonyl-1,4-diaza-12-tert-butoxycarbonyl-2-oxobicyclo[7.3.0]dodec-6-ene **19**

To a degassed solution of diene **17** (0.10 g, 0.23 mmol) in dry dichloromethane (56 cm³) was added

5 bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs's catalyst) (0.018 g, 0.023 mmol) under an atmosphere of nitrogen and the resultant purple solution heated at reflux for 24 h. Further bis(tricyclohexylphosphine)benzylidineruthenium dichloride (0.018 g, 0.023 mmol) was added and refluxing continued for 24 h, then the reaction mixture

10 was cooled to room temperature and dimethyl sulphoxide (0.160 cm³, 2.3 mmol) was added and the orange/brown solution stirred for 24 h. The solvent was removed *in vacuo* and the residue purified by chromatography (SiO₂, 3:1, 2:1, 1:1, hexanes-ethyl acetate,) to give an almost colourless oil which was further purified by chromatography (C₁₈ RP silica, 100:0, 9:1, 9:3, 1:1, 1:9, water-acetonitrile,) to give *alkene 19* [0.039 g, 42% (60% based on recovered starting material)] as a colourless oil. Alkene **19** existed exclusively as the *trans* C(O)-NPro conformer. In addition, restricted rotation about the N-CO carbamate bond was also observed resulting in a 1:1 mixture of conformers:

20 [α]_D -118.2 (*c* 0.34 in CH₂Cl₂); δ_H (400 MHz; CDCl₃; Me₄Si) 1.46 [9H, s, C(CH₃)₃], 1.74 (1H, dd, *J* 11.8 and 5.7, 10-*H_AH_B*), 1.93 (1H, dd, *J* 12.5 and 6.5, 11-*H_AH_B*), 2.11-2.45 (4H, m, 8-H₂, 10-*H_AH_B* and 11-*H_AH_B*), 3.53 (1H, dd, *J* 15.7 and 8.0, 5-*H_AH_B*), 4.13-4.37 (4H, m, 3-H₂, 9-H and 12-H), 4.50 (1H, d, *J* 15.4, 5-*H_AH_B*), 5.12-5.29 (2H, m, OCH₂Ph), 5.54-5.82 (2H, m, 6-H and 7-H) and 7.31-7.43 (5H, m, Ph); δ_C (100 MHz; CDCl₃) 26.7 (CH₂, 11-C), 27.8 [CH₃, C(CH₃)₃], 34.0 (CH₂), 34.2 (CH₂), 34.3 (CH₂), 34.4 (CH₂), 43.9 (CH₂, 5-C), 44.5 (CH₂, 5-C), 48.5 (CH₂, 3-C), 49.5 (CH₂, 3-C), 57.2 (CH, 9-C), 57.6 (CH, 9-C), 60.1 (CH, 12-C), 67.6 (CH₂, OCH₂Ph), 81.2 [quat., C(CH₃)₃], 126.8 (CH, 6-C), 126.9 (CH, 6-C), 128.0 (CH, Ph), 128.4 (CH, Ph), 128.7 (CH, Ph), 129.9 (CH, 7-C), 136.3 (quat., Ph), 136.4 (quat., Ph), 155.7 (quat., NCO₂), 167.8

25

(quat., 2-C), 167.9 (quat., 2-C) and 170.7 (quat., 12-CO); m/z (EI+) 414.2157 (M^+ . $C_{23}H_{30}N_2O_5$ requires 414.2155).

5 *(9S, 12S)-1,4-Diaza-4-tert-butyloxycarbonyl-2-oxo-bicyclo[7.3.0]dodecyl-12-carboxylic acid 20*

To a stirred solution of alkene **19** (0.11 g, 0.263 mmol) in tetrahydrofuran (4 cm³) was added platinum oxide (0.006 g, 0.026 mmol) under a flow of nitrogen. The mixture was hydrogenated (1 atm. of hydrogen) for 16 h, filtered over Celite™, and the solvent removed *in vacuo*. The residue was
 10 dissolved in a solution of 30% hydrobromic acid in acetic acid (3 cm³) and stirred at room temperature for 2 h. Removal of the volatiles *in vacuo* at 40 °C followed by repeated evaporation from methanol:water (3:1) yielded the hydrobromide salt which was dissolved in a solution of saturated sodium hydrogen carbonate (3 cm³). Dioxane (2 cm³) and di-*tert*-butyl dicarbonate
 15 (0.07 g, 0.32 mmol) were added and the resultant suspension was stirred for 24 h, then further di-*tert*-butyl dicarbonate (0.07 g, 0.32 mmol) was added and stirring continued for a further 48 h. Water (10 cm³) was added, the solution washed with dichloromethane and the aqueous layer was acidified with 2 M aqueous hydrochloric acid and extracted with dichloromethane. The combined
 20 organic layers were dried (Na₂SO₄), filtered and the solvent removed to yield an oil (0.072 g) that was purified by chromatography (SiO₂, 1:1:0.3, hexanes-ethyl acetate:acetic acid) to give *acid 20* (0.05 g, 58%) as a colourless oil. *Acid 20* existed exclusively as the *trans* C(O)-NPro conformer. In addition, restricted rotation about the N-CO carbamate bond was also observed resulting in a 72:28
 25 mixture of conformers: $[\alpha]_D +3.5-4.4$ (c 0.23 in CH₂Cl₂); δ_H (400 MHz; CDCl₃; Me₄Si) 1.30-1.48 [11H, m, C(CH₃)₃, 8- H_AH_B and 11- H_AH_B], 1.63-1.79 (5H, m, 8- H_AH_B , 6-H₂ and 7-H₂), 2.01-2.07 (1H, m, 11- H_AH_B), 2.12-2.32 (2H, m, 10-H₂), 2.62-2.85 (1H, m, 5- H_AH_B), 3.73-3.83 (1.28 H, 3- H_AH_B and 5- $H_AH_B^*$), 4.0

(0.72H, br d, J 14.2, 5- H_AH_B), 4.20-4.32 (1H, m, 9-H), 4.41* (0.28H, d, J 8.0, 12-H), 4.50 (0.72H, d, J 8.8, 12-H), 4.57 (0.72H, d, J 17.8, 3- H_AH_B), 4.69* (0.28H, d, J 16.5, 3- H_AH_B) and 8.40 (1H, br s, OH); δ_C (100 MHz; $CDCl_3$) 22.1 (CH₂, 7-C), 22.6* (CH₂, 7-C), 25.1 (CH₂, 11-C), 25.7* (CH₂, 11-C), 26.6 (CH₂, 6-C), 28.1 [CH₃, C(CH₃)₃], 28.3* [CH₃, C(CH₃)₃], 32.5 (CH₂, 10-C), 32.7* (CH₂, 10-C), 35.9* (CH₂, 8-C), 36.3 (CH₂, 8-C), 50.3* (CH₂, 5-C), 51.8 (CH₂, 5-C), 54.9* (CH₂, 3-C), 55.4 (CH, 9-C), 56.0* (CH, 9-C), 56.3 (CH₂, 3-C), 60.5* (CH, 12-C), 60.8 (CH, 12-C), 80.6* [quat., C(CH₃)₃], 81.0 [quat., C(CH₃)₃], 155.2 (quat., NCO₂), 155.5* (quat., NCO₂), 169.5* (quat., 2-C), 169.8 (quat., 2-C), 174.9 (quat., 12-CO) and 175.1* (quat., 12-CO); m/z (EI+) 326.1837 (M^+ . C₁₆H₂₆N₂O₅ requires 326.1842).

(2*S*, 9'*S*, 12'*S*)-2-{[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt **22**

15 Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoP-Cl) (0.057 g, 0.225 mmol) was added to a solution of acid **10** (0.05 g, 0.15 mmol), L-glutamic acid di-*tert*-butyl ester hydrochloride **21** (0.062 g, 0.21 mmol) and triethylamine (0.061 cm³, 0.44 mmol) in dichloromethane (5 cm³) at 0 °C. The mixture was stirred for 17 h, washed with saturated aqueous sodium hydrogen carbonate, 2
20 M aqueous hydrochloric acid, dried (Na₂SO₄), filtered and the solvent removed to yield an oil (0.108 g) which was purified by chromatography (SiO₂, 1:1, 1:2, 1:3, hexane-ethyl acetate) to afford an inseparable mixture (0.074 g) of the desired amide contaminated with a glutamate/bis(2-oxo-3-oxazolidinyl)phosphinic chloride adduct. The mixture was dissolved in
25 dichloromethane (5 cm³), trifluoroacetic acid (2 cm³) was added and the solution stirred for 4 h. Further trifluoroacetic acid (1 cm³) was then added and stirring continued for 2 h. The volatiles were removed *in vacuo*, the residue suspended in water and filtered through a plug of cotton wool. The filtrate was subsequently purified by RP-HPLC [water (0.05% trifluoroacetic

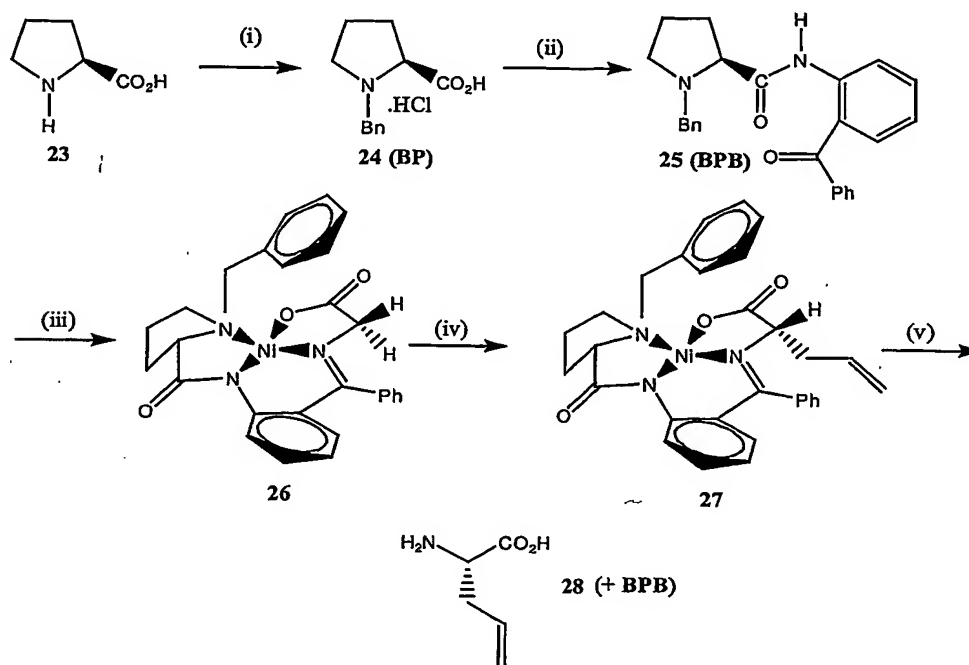
acid):acetonitrile, 90:10, 13 ml min⁻¹] to afford trifluoroacetate **22** (0.039 g, 55% from **20**) as a white solid after titration from diethyl ether/toluene. Macrocyclic **22** existed exclusively as the *trans* C(O)-NPro conformer: mp 50-100 °C; [α]_D -22.3 (c 0.35 in MeOH); δ_H (400 MHz; CDCl₃; Me₄Si) 1.68-2.04 (9H, m, 7'-H₂, 6'-H₂, 10'-H₂, 3'-H_AH_B, 8'-H_AH_B and 11'-H_AH_B), 2.19-2.26 (2H, 8'-H_AH_B and 3'-H_AH_B), 2.42-2.48 (1H, m, 11'-H_AH_B), 2.50-2.58 (2H, m, 4-H₂), 3.04 (1H, td, *J* 11.2 and 2.5, 5'-H_AH_B), 3.33 (1H, dt, *J* 14.1 and 4.9, 5'-H_AH_B), 3.82 (1H, d, *J* 14.1, 3'-H_AH_B), 4.32 (1H, d, *J* 14.0, 3'-H_AH_B), 4.38-4.45 (2H, m, 9'-H and 2-H) and 4.59 (1H, dd *J* 9.6 and 1.8, 12'-H); δ_C (100 MHz; CDCl₃) 25.6 (CH₂, 6'-C or 7'-C), 26.0 (CH₂, 6'-C or 7'-C), 28.1 (CH₂, 3-C), 29.9 (CH₂, 11'-C), 32.3 (CH₂, 4-C), 34.0 (CH₂, 10'-C or 8'-C), 35.2 (CH₂, 10'-C or 8'-C), 46.9 (CH₂, 5'-C), 48.7 (CH₂, 3'-C), 54.3 (CH, 2-C), 62.9 (CH, 12'-C), 64.6 (CH, 9'-C), 118.6 (quat., q, *J* 291, CF₃), 165.1 (quat., q, *J* 36.2, CF₃CO₂H), 167.9 (quat., 2'-C), 175.9 (quat., 12'-CO), 177.1 (quat., 1-C), and 179.4 (quat., 5-C); *m/z* (FAB⁺) 356.1824 [MH(free base)⁺. C₁₆H₂₆N₃O₆ requires 356.1822].

20

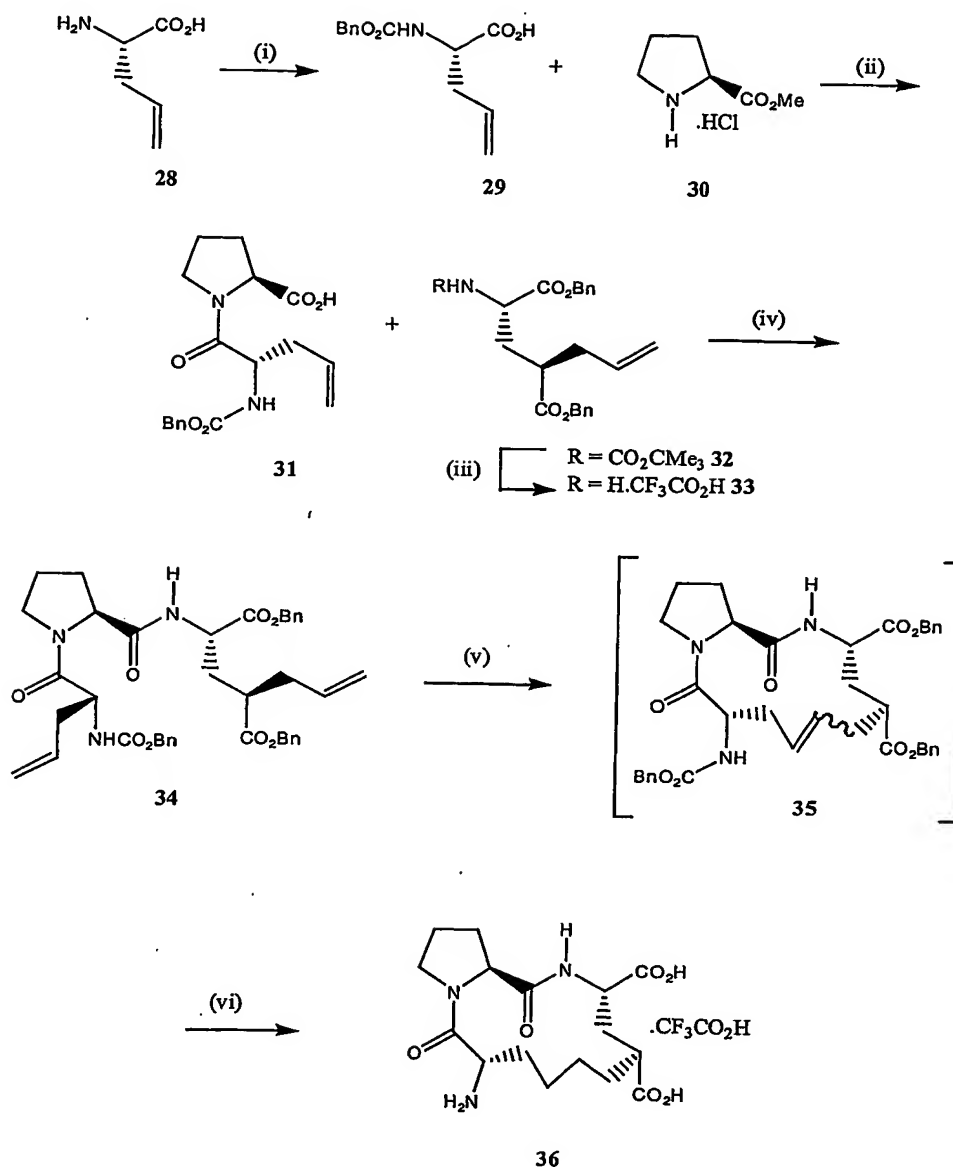
25

30

Example 3: Synthesis of (3*S*, 8*R*, 10*S*, 13*S*) 3-amino-1,11-diaza-8,10-carboxy-2,12-oxobicyclo[13.3.0]hexadecane trifluoroacetate



Scheme 1 Reagents, conditions and yields: (i) KOH, BnCl, ⁱPrOH, 40 °C, 5.5 h (78%); (ii) SOCl₂, CH₂Cl₂, Et₃N -30 °C, then 2-aminobenzophenone, RT, overnight (43%); (iii) KOH, glycine, Ni(NO₃)₂·6H₂O, 60 °C, 75 min (78%); (iv) NaOH, allyl bromide, CH₃CN, 2.5 h, RT (68%); (v) 2M aq. HCl, MeOH, 80 °C 1 h, (84%).



Scheme 2 Reagents, conditions and yields: (i) BnOCOCl , Na_2CO_3 , H_2O /dioxane, 0°C to RT, 17 h (53%); (ii) DCC, HOBT, Et_3N , CH_2Cl_2 , 0°C to RT, overnight, then 1 M aq. NaOH, dioxane, RT, 24 h (64% over 2 steps); (iii) TFA, CH_2Cl_2 , 0°C 2 h (iv) 31, EtOCOCl , Et_3N , CH_2Cl_2 , 0°C , 40 min, then 33 0°C to RT overnight (60%); (v) Grubbs's catalyst, CH_2Cl_2 , reflux, 48 h then DMSO, 24 h; (vi) 10% Pd/C, H_2 , THF/ H_2O (4:1), RT, 21 h or 10% PtO_2 , H_2 , THF, RT, 16 h then 10% Pd/C, H_2 , MeOH/ H_2O (4:1), RT, 5 h (58% from 34).

N-Benzyl-L-proline hydrochloride 24 (BP)

A suspension L-proline 23 (6.0 g, 52.1 mmol) and potassium hydroxide (85%, 11.1 g, 198 mmol) in isopropanol (50 cm³) was heated at 40 °C until mostly dissolved. Benzyl chloride (9.2 cm³, 79.7 mmol) was added dropwise during which the internal temperature rose to 70 °C. The addition was stopped, the reaction flask placed in an ice bath until an internal temperature of 40 °C had been obtained, and then the addition continued at 40 °C. The milky-white suspension was stirred at 40 °C for 5.5 h, cooled to room temperature and neutralised with 32% hydrochloric acid until pH=4 had been obtained. Chloroform (50 cm³) was added, the suspension stored overnight at 0 °C and the white solid was removed by filtration. The filtrate was concentrated *in vacuo*, suspended in acetone, the solid filtered and air dried to give BP 24 (9.842 g, 78%) as a white solid: mp 173-176 °C (dec) (lit., 174-175, free base) [α]_D -22.9 (*c* 0.20 in H₂O) (lit.,¹ -25.8 *c* 1 in EtOH, free base); δ_H (300 MHz; D₂O) 1.91-2.21 (3H, Pro γ -H₂, Pro β -H_AH_B), 2.47-2.60 (1H, m, Pro β -H_AH_B), 3.27-3.36 (1H, m, Pro δ -H_AH_B), 3.60-3.67 (1H, m, Pro δ -H_AH_B), 4.14 (1H, dd, *J* 9.3 7.0, Pro α -H), 4.37 (1H, d, *J* 12.9, NCH_AH_BPh), 4.44 (1H, d, *J* 12.9, NCH_AH_BPh) and 7.45-7.54 (5H, m, Ph); δ_C (75 MHz; D₂O) 22.4 (CH₂, Pro γ -C), 28.4 (CH₂, Pro β -C), 54.6 (CH₂, Pro δ -C), 58.3 (CH₂, NCH₂Ph), 67.1 (CH, Pro α -C), 129.0, 130.4 (CH, Ph), 129.6 (quat., Ph), 129.9 (CH, Ph) and 172.4 (quat., Pro α -CO).

(*S*)-2-[*N*-(*N*'-Benzylprolyl)amino]benzophenone 25 (BPB)

To an opaque solution of BP 24 (10.567 g, 43.84 mmol) and triethylamine (5.9 cm³, 43.84 mmol) in dry dichloromethane (70 cm³) was added thionyl chloride (4.3 cm³, 58.5 mmol) over 5 min at -30 °C. The resultant orange suspension was allowed to warm to -10 °C over 30 min, stirred for 30 min at this temperature and cooled to -30 °C. A solution of 2-aminobenzophenone (5.76 g, 29.2 mmol) in dry dichloromethane (30 cm³) was

added over 5 min and the yellow-orange solution stirred at room temperature overnight. The mixture was cooled to 0 °C and a solution of sodium carbonate (9.1 g) in water (50 cm³) was added carefully. After the evolution of gas had ceased, the organic layer was removed, the aqueous layer extracted with dichloromethane, and combined extracts dried (MgSO₄). Evaporation of the solvent and initial purification by chromatography (silica gel, hexanes:ether, 4:1, 3:1, 1:1) [to remove most of 2-aminobenzophenone] gave a solid (6.405 g) which by ¹H NMR contained 10% 2-aminobenzophenone. Recrystallisation from boiling hexanes afforded *BPB* **25** (5.218 g, 46%) as yellow crystals: mp 99-102 °C (lit., 101-102 °C); [α]_D -129.1 (c 0.12 in MeOH) (lit., -134 c 1 in MeOH); δ_H (300 MHz; CDCl₃; Me₄Si) 1.78-2.04 (3H, Pro γ -H₂, Pro β -H_AH_B), 2.21-2.47 (2H, m, Pro β -H_AH_B, Pro δ -H_AH_B), 3.22-3.24 (1H, m, Pro δ -H_AH_B), 3.34 (1H, dd, *J* 10.1 and 4.6, Pro α -H), 3.61 (1H, d, *J* 12.9, NCH_AH_BPh), 3.94 (1H, d, *J* 12.9, NCH_AH_BPh), 7.08-7.65 (12H, m, Ph), 7.81 (2H, d, *J* 8.2, Ph) and 11.6 (1H, s, N-H); δ_C (75 MHz; CDCl₃) 24.1 (CH₂, Pro γ -C), 30.9 (CH₂, Pro β -C), 53.8 (CH₂, Pro δ -C), 59.8 (CH₂, NCH₂Ph), 68.2 (CH, Pro α -C), 121.4, 122.7 (CH, Ph), 125.2 (quat., Ph), 127.1, 128.1, 128.2, 129.0, 130.0, 132.4, 132.5, 133.3 (CH, Ph), 138.0, 138.5, 139.0 (quat., Ph), 174.6 (quat., Pro-CON) and 198.0 (quat., Ph₂CO).

20

Gly-Ni-BPB complex 26

A solution of potassium hydroxide (4.56 g, 81.3 mmol) in methanol (30 cm³) was added to a suspension of *BPB* **25** (4.46 g, 11.61 mmol), glycine (4.36 g, 58.07 mmol) and nickel nitrate hexahydrate (6.93 g, 23.2 mmol) at 50 °C under an atmosphere of nitrogen. The mixture was heated at 60 °C for 75 min, cooled to room temperature and acetic acid (4.65 cm³, 81.3 mmol) added. Water (150 cm³) was then added and the mixture allowed to stand at room temperature for 2 h. Extraction of the product with dichloromethane yielded a blood-red syrup which was purified by chromatography (silica gel, dichloromethane:acetone, 7:1, 6:1, 4:1, 3:1, 2:1) to give the crude product which

30

was recrystallised from ethyl acetate at -20 °C to give *complex 26* (4.377 g, 78%) as a red solid: mp 226-228 °C (lit., 208-212 °C); $[\alpha]_D^{20} +2010.6$ (*c* 0.104 in MeOH) (lit., +2006 *c* 0.1 in MeOH); δ_H (400 MHz; CDCl₃; Me₄Si) 2.03-2.20 (2H, Pro γ -H_AH_B, Pro δ -H_AH_B), 2.38-2.49 (1H, m, Pro β -H_AH_B), 2.55 (1H, m, Pro β -H_AH_B), 3.30-3.42 (1H, m, Pro γ -H_AH_B), 3.48 (1H, dd, *J* 10.7 and 5.4, Pro α -H), 3.66-3.75 (3H, m, Pro δ -H_AH_B, NCH_AH_BPh, Gly α -H_AH_B), 3.79 (1H, d, *J* 20.1, Gly α -H_AH_B), 4.49 (1H, d, *J* 12.9, NCH_AH_BPh), 6.71 (1H, t, *J* 7.2, Ph), 6.81 (1H, dd, *J* 8.2 and 1.5, Ph), 6.93-7.00 (1H, m, Ph), 7.11 (1H, br d, *J* 7.2, Ph), 7.20-7.24 (1H, m, Ph), 7.32 (1H, t, *J* 7.5, Ph), 7.44 (2H, t, *J* 7.52, Ph), 7.51-7.57 (3H, m, Ph), 8.09 (2H, d, *J* 7.4, Ph) and 8.30 (1H, d, *J* 8.6, Ph); δ_C (100 MHz; CDCl₃) 23.6 (CH₂, Pro γ -C), 30.6 (CH₂, Pro β -C), 57.4 (CH₂, Pro δ -C), 61.2 (CH₂, Gly α -C), 63.1 (CH₂, NCH₂Ph), 68.9 (CH, Pro α -C), 120.8, 124.2 (CH, Ph), 125.1 (quat., Ph), 125.6, 126.2, 128.8, 129.0, 129.3, 129.5, 129.6, 131.6, 132.1, 133.1 (CH, Ph), 133.3, 134.5, 142.5 (quat., Ph), 171.5 (quat., C=N), 177.7 (quat., Gly α -CO) and 181.3 (quat., Pro-CON).

AllylGly-Ni-BPB complex 27

Powdered sodium hydroxide (0.733 g, 18.34 mmol) and allyl bromide (2.06 cm³, 22.0 mmol) was added to a stirred solution of *complex 26* (3.6 g, 7.33 mmol) in dry acetonitrile (160 cm³) under a nitrogen atmosphere. The mixture was stirred for 2.5 h at room temperature, 0.1M aqueous hydrochloric acid (100 cm³) added and the product extracted with dichloromethane. The combined organic layers were dried (MgSO₄), the solvent removed and the residue purified by chromatography (silica gel, dichloromethane:acetone, 2:1) to give a mixture of diastereomers (1.468 g) and one diastereomer (2.174 g). Both fractions were recrystallised from ethyl acetate at -20 °C to give the desired compound (1.9 g); further compound was obtained from crystallization from the mother liquors (0.766 g) to give *allyl complex 27* (2.676 g, 68%) as a red solid (single diastereomer): mp 216-219 °C (lit., 217 °C); δ_H (300 MHz; CDCl₃;

Me₄Si) 2.0-2.16 (2H, m, Proγ-H_AH_B, Proδ-H_AH_B), 2.36-2.55 [3H, m, Proβ-H_AH_B, CH₂(allyl)], 2.74-2.83 (1H, m, Proβ-H_AH_B), 3.41-3.61 (4H, m, Proγ-H_AH_B, Proα-H, Proδ-H_AH_B, NCH_AH_BPh), 4.01 (1H, dd, *J* 6.3 and 4.1, Glyα-H), 4.42 (1H, d, *J* 12.6, NCH_AH_BPh), 5.17 (1H, d, *J* 17, =CH_AH_B), 5.38 (1H, d, *J* 10, =CH_AH_B), 6.36-6.50 (1H, m, C(H)=CH₂), 6.61-6.68 (2H, m, Ph), 6.94 (1H, d, *J* 7.0, Ph), 7.11-7.54 (8H, m, Ph), 8.03 (2H, d, *J* 7.4, Ph) and 8.17 (1H, d, *J* 8.7, Ph); δ_C (75 MHz; CDCl₃) 23.3 (CH₂, Proγ-C), 30.8 (CH₂, Proβ-C), 38.5 [CH₂, CH₂(allyl)], 56.9 (CH₂, Proδ-C), 63.1 (CH₂, NCH₂Ph), 70.27, 70.34 (CH, Proα-C, Glyα-C), 119.7 (CH₂, =CH₂), 120.6, 123.6 (CH, Ph), 126.4 (quat., Ph), 127.0, 127.7, 128.8, 128.9, 129.0, 129.8, 131.5, 132.1, 132.2, (CH, C(H)=CH₂, Ph), 133.2, (quat., Ph), 133.3 (CH, Ph), 133.9, 142.5 (quat., Ph), 170.8 (quat., C=N), 178.8 (quat., Glyα-CO) and 180.3 (quat., Pro-CON).

(*S*)-Allylglycine 28

15 A mixture of allyl complex 27 (2.520 g, 4.69 mmol) and 2M aqueous hydrochloric acid (54 cm³) in methanol (78 cm³), was heated (oil bath 80 °C) for 1 h. The light green solution was cooled to room temperature and 28% ammonia solution was added until pH=9-10. The aqueous layer was extracted with dichloromethane to recover BPB (1.82 g, 100% recovery) and the aqueous
20 layer was concentrated *in vacuo* to afford a blue solid that was purified by ion exchange chromatography (Dowex 50Wx8-100, H₂O, then 5% ammonia solution). The ammonia fractions were combined, evaporated to dryness, suspended in toluene and concentrated *in vacuo* (2x) to yield a solid that was dried on a freeze drier to give allylglycine 28 (0.455 g, 84%) as a white
25 powder: mp 195-190 °C (dec.) (lit., 208 °C); [α]_D -40.5 (c 0.11 in H₂O) (lit., -42.7 c 4 in H₂O); δ_H (400 MHz; D₂O) 2.62-2.76 [2H, m, CH₂(allyl)], 3.87 (1H, dd, *J* 6.9 and 5.4, Glyα-H), 5.30-5.35 (2H, m, =CH₂) and 5.58-5.88 (1H, m, C(H)=CH₂); δ_C (100 MHz; D₂O) 34.7 [CH₂, CH₂(allyl)], 53.8 (CH, Glyα-C), 120.4 (CH₂, =CH₂), 131.2 (CH, C(H)=CH₂) and 174.0 (quat., Glyα-CO).

(S)-N-Benzylloxycarbonylallylglycine 29

A solution of benzyl chloroformate (0.135 cm³, 0.956 mmol) in dioxane (1 cm³) was added to a solution of allylglycine **28** (0.1 g, 0.870 mmol) and sodium carbonate (0.275 g, 2.60 mmol) in water (5 cm³) at 0 °C. The solution was allowed to warm to room temperature over 2 h, then stirred for a further 17 h. The aqueous layer was washed with ether, acidified with 31% hydrochloric acid to pH=1 and extracted with ethyl acetate. The pooled organic layers were dried (MgSO₄) and the solvent removed *in vacuo* to give carbamate **7** (0.114 g, 53%) as an opaque oil. Carbamate **29** was shown to be a 74:26 mixture of rotamers: δ_{H} (300 MHz; CDCl₃; Me₄Si) 2.52-2.67 [2H, m, CH₂(allyl)], 4.37* (0.26H, br s, Gly α -H), 4.46-4.52 (0.76H, m, Gly α -H), 5.08-5.18 (4H, m, OCH₂Ph, =CH₂) 5.48 (0.74H, d, *J* 7.9, N-H), 5.69-5.79 (1H, m, C(H)=CH₂), 6.34 (0.26H, br d, *J* 5.6, N-H), 7.26-7.34 (5H, m, Ph) and 9.46 (1H, br s, OH); δ_{C} (75 MHz; CDCl₃) 36.4 [CH₂, CH₂(allyl)], 53.2 (CH, Gly α -C), 53.9* (CH, Gly α -C), 67.2 (CH₂, OCH₂Ph), 67.7* (CH₂, OCH₂Ph), 119.6 (CH₂, =CH₂), 128.1, 128.3, 128.6 (CH, Ph), 131.9 (CH, C(H)=CH₂), 136.1 (quat., Ph), 156.1 (quat., NCO₂) and 176.0 (quat., Gly α -CO).

(S)-N-Benzylloxycarbonylallylglycyl-L-proline 31

N,N-Dicyclohexylcarbodiimide (0.103 g, 0.504 mmol) was added to a solution of carbamate (0.114 g, 0.457 mmol), proline methyl ester hydrochloride **30** (0.083 g, 0.504 mmol), *N*-hydroxybenzotriazole (0.068 g, 0.504 mmol) and triethylamine (0.071 cm³, 0.504 mmol) in dichloromethane (12 cm³) at 0 °C. The mixture was stirred overnight, stored at 0 °C for 3 h and filtered thorough Celite™. The reaction mixture was washed with 2M aqueous hydrochloric acid, saturated sodium hydrogen carbonate solution, dried (MgSO₄) and the solvent removed to yield an oil which was purified by

chromatography (silica gel, hexanes:ethyl acetate, 2:1, 1:1) to afford *protected dipeptide* (0.141 g) that was suspended in dioxane (4 cm³). 1M Aqueous sodium hydroxide (1.95 cm³, 1.95 mmol) was added and the mixture stirred at room temperature for 24 h. Water (5 cm³) was added and the mixture extracted with dichloromethane. The aqueous layer was acidified with 31% hydrochloric acid and the product extracted with dichloromethane. The organic layers were pooled, washed with brine, dried (MgSO₄) and the solvent removed to afford an oil (0.152 g) that was purified by chromatography (silica gel, hexane:ethyl acetate, 3:1, then hexane:ethyl acetate:acetic acid, 3:1:0.4, 2:1:0.3) to give *acid* 31 (0.102 g, 64%) as a colourless oil. Acid 31 was shown to be a 87:13 *trans:cis* mixture of conformers by ¹H NMR analysis (the ratio was estimated by integration of multiplets at δ 3.33-3.50 and 3.62-3.77 assigned to the Pro δ -H₂ atoms of the minor and major conformers respectively): [α]_D -91 (*c* 0.26 in CH₂Cl₂); δ _H (300 MHz; CDCl₃; Me₄Si) 1.65* (0.13H, br s, Pro β -H_AH_B), 1.84-2.25 (3.87H, m, Pro β -H_AH_B,* Pro β -H₂, Pro γ -H₂), 2.34-2.53 [2H, m, CH₂(allyl)], 3.33-3.50* (0.26H, m, Pro δ -H₂), 3.62-3.77 (1.74H, m, Pro δ -H₂), 4.30-4.58 (2H, m, Pro α -H, Gly α -H), 4.93-5.15 (4H, m, OCH₂Ph, =CH₂) 5.71-5.85 (1H, m, C(H)=CH₂), 6.0 (0.13H, br d, *J* 8.5, N-H), 6.06* (0.13H, br d, *J* 8.7, N-H), 7.26-7.31 (5H, m, Ph) and 8.01 (1H, br s, OH); δ _C (75 MHz; CDCl₃) 22.1* (CH₂, Pro γ -C), 24.8 (CH₂, Pro γ -C), 28.6 (CH₂, Pro β -C), 31.2* (CH₂, Pro β -C), 36.6 [CH₂, CH₂(allyl)], 37.8* [CH₂, CH₂(allyl)], 46.7* (CH₂, Pro δ -C), 47.3 (CH₂, Pro δ -C), 52.1 (CH, Gly α -C), 52.3* (CH, Gly α -C), 59.2 (CH, Pro α -C), 66.6 (CH₂, OCH₂Ph), 67.1* (CH₂, OCH₂Ph), 119.1 (CH₂, =CH₂), 127.9, 128.0, 128.4, 128.5 (CH, Ph), 132.4 (CH, C(H)=CH₂), 136.1* (quat., Ph), 136.3 (quat., Ph), 156.1 (quat., NCO₂), 170.8* (quat., Gly α -CO), 171.4 (quat., Gly α -CO) and 174.6 (quat., Pro α -CO); *m/z* (EI⁺) 346.1527 (M⁺. C₁₈H₂₂N₂O₅ requires 346.1529).

(S)-N-Benzoyloxycarbonylallylglycyl-L-prolyl-L-γ(R)-allylglutamic acid dibenzyl ester 34

Alkene **32** (0.217 g, 0.464 mmol) was dissolved in dichloromethane (7 cm³), cooled to 0 °C and trifluoroacetic acid (1.5 cm³) was added. After stirring for 2 h the volatiles were removed *in vacuo* to yield *trifluoroacetate* **33** as an off white solid. To an ice cold solution of acid **31** (0.134 g, 0.387 mmol) and triethylamine (0.062 cm³, 0.461 mmol) in dichloromethane (10 cm³) was added dropwise ethyl chloroformate (0.045 cm³, 0.464 mmol). The solution was stirred at 0 °C for 40 min then an ice cold solution of **33** and triethylamine (0.062 cm³, 0.464 mmol) in dichloromethane (5 cm³) was added dropwise and the mixture stirred overnight. The solution was subsequently washed with 2M aqueous hydrochloric acid, saturated sodium hydrogen carbonate solution, dried (MgSO₄) and the solvent removed to yield an oil which was purified by chromatography (silica gel, hexane:ethyl acetate, 2:1) to give *protected tripeptide* **34** (0.162 g, 60%) as a colourless oil. Tripeptide **34** was shown to be a 89:11 *trans:cis* mixture of conformers by ¹H NMR analysis (the ratio was estimated by integration of the doubles at δ 8.04 and 7.10 assigned to the GluN-H atoms of the minor and major conformers respectively): [α]_D -31.2 (c 0.462 in CH₂Cl₂); δ_H (400 MHz; CDCl₃; Me₄Si) 1.90-1.99 (2H, m, Proβ-H_AH_B, Proγ-H_AH_B), 2.06-2.13 (3H, m, Proγ-H_AH_B, Gluβ-H₂), 2.19-2.26 (1H, m, Proβ-H_AH_B), 2.33-2.42 [3H, m, CH₂(allyl), CH₂CH₂(allyl)], 2.47-2.54 (1H, m, CH₂CH₂(allyl)), 2.63 (1H, p, *J* 6.7, Gluγ-H), 3.56-3.72 (2H, m, Proδ-H₂), 4.18* (0.11H, q, *J* 6.2, Glyα-H), 4.38* (0.11H, d, *J* 7.7, Proα-H), 4.54-4.66 (2.78H, Glyα-H, Proα-H), Gluα-H), 4.86-5.18 (10H, m, 3 x OCH₂Ph, 2 x =CH₂), 5.60-5.81 (3H, m, 2 x C(H)=CH₂, GlyN-H), 7.10 (0.89H, d, *J* 7.4, GluN-H), 7.26-7.39 (15H, m, Ph) and 7.10* (0.11H, d, *J* 7.9, GluN-H); δ_C (100 MHz; CDCl₃) 22.0* (CH₂, Proγ-C), 24.9 (CH₂, Proγ-C), 27.5 (CH₂, Proβ-C), 31.3* (CH₂, Proβ-C), 31.4* (CH₂, Gluβ-C), 32.9 (CH₂, Gluβ-C), 35.65* [CH₂, CH₂(allyl)], 35.71* [CH₂, CH₂(allyl)], 35.9 [CH₂, CH₂(allyl)], 36.9 [CH₂, CH₂(allyl)], 41.0 (CH,

Glu γ -C), 41.9* (CH, Glu γ -C), 46.9* (CH₂, Pro δ -C), 47.3 (CH₂, Pro δ -C), 50.7
 (CH, Glu α -C), 51.3* (CH, Glu α -C), 51.8 (CH, Gly α -C), 52.4* (CH, Gly α -C),
 59.9 (CH, Pro α -C), 60.8* (CH, Pro α -C), 66.31* (CH₂, OCH₂Ph), 66.4 (CH₂,
 OCH₂Ph), 66.8 (CH₂, OCH₂Ph), 67.0* (CH₂, OCH₂Ph), 67.2 (CH₂, OCH₂Ph),
 5 117.7, 119.0, 119.8* (CH₂, =CH₂), 127.9, 128.0, 128.14, 128.22, 128.24,
 128.31, 128.42, 128.46, (CH, Ph), 131.7,* 132.2, 134.2 (CH, C(H)=CH₂), 135.2,
 135.3,* 135.7, 136.0,* 136.2 (quat., Ph), 155.8 (quat., NCO₂), 156.2* (quat.,
 NCO₂), 170.7* (quat., CO), 170.9 (quat., CO) 171.2 (quat., CO), 171.5 (quat.,
 CO), 171.6* (quat., CO), 174.3* (quat., Glu γ -CO) and 174.4 (quat., Glu γ -CO);
 10 *m/z* (EI⁺) 695.3170 (M⁺. C₄₀H₄₅N₃O₈ requires 695.3207).

(3*S*, 8*R*, 10*S*, 13*S*) 3-Amino-1,11-diaza-8,10-carboxy-2,12-
oxobicyclo[13.3.0]hexadecane trifluoroacetate 36

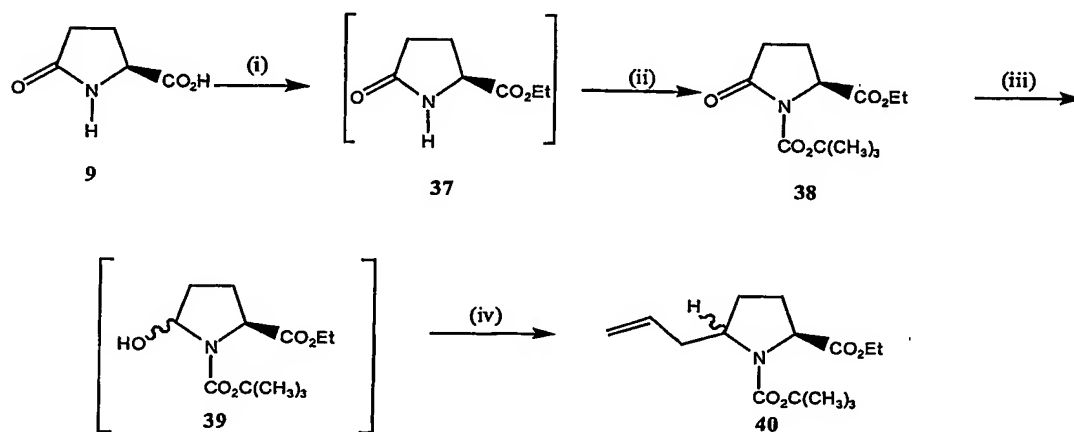
To a solution of protected tripeptide **34** (0.041 g, 0.0589 mmol) in dry
 15 dichloromethane (7.5 cm³) was added bis(tricyclohexylphosphine)-
 benzyldineruthenium dichloride (0.015 g, 0.0126 mmol) under an atmosphere
 of nitrogen. The purple solution was heated at reflux for 48 h, cooled to room
 temperature and dimethyl sulphoxide (0.045 cm³, 0.63 mmol) added. The
 orange-brown solution was stirred for 24 h, filtered through a short plug of
 20 silica gel (eluting with hexanes:ethyl acetate, 1:3), and purified by
 chromatography (C₁₈ RP silica, water:acetonitrile, 20%-70%) to give the
 metathesis product **35** (48 mg) and other unidentified product(s) as a yellow oil,
 [*m/z* (FAB⁺) 668.2977 (MH⁺. C₃₈H₄₂N₃O₈ requires 668.2972)]. Metathesis
 product was divided into 2 samples (~25 mg each); the first sample was
 25 dissolved in tetrahydrofuran/water (4:1, 5 cm³) and 10 wt. % palladium on
 activated carbon (0.008 g, 0.00749 mmol) was added under an atmosphere of
 nitrogen. To the reaction flask was fitted a balloon of hydrogen and stirred for
 21 h at room temperature. The reaction mixture was filtered through a pad of
 CeliteTM, washed with methanol/water (4:1) and concentrated *in vacuo* to yield a

film. The second sample was dissolved in tetrahydrofuran (3 cm³) and platinum (IV) oxide (0.00078 g, 0.0034 mmol) added was added under an atmosphere of nitrogen. To the reaction flask was fitted a balloon of hydrogen and stirred for 16 h at room temperature. The reaction mixture was filtered through a pad of
5 Celite™, washed with methanol: and concentrated *in vacuo* to yield a film that was dissolved in methanol:water (4:1, 5 cm³) and 10 wt. % palladium on activated carbon (0.008 g 0.00749 mmol) was added under an atmosphere of nitrogen. To the reaction flask was fitted a balloon of hydrogen and stirred for 5 h at room temperature. The reaction mixture was filtered through a pad of
10 Celite™, washed with methanol/water (4:1) and concentrated *in vacuo* to yield a film. Both samples were combined, purified by RP HPLC [10% acetonitrile:90% water (containing 0.05% trifluoroacetic acid) then 80:20, 70:30] and dried on a freeze drier to give 36 (0.016 g, 58% from 34) as a white solid. 36 existed exclusively as the *trans* conformer: mp 150-240 °C; [α]_D -39.4
15 (c 0.0864 in water); δ_H (400 MHz; D₂O), 1.20-1.62 (6H, m, 5-H₂, 14-H₂, 7-H₂), 1.81-1.90 (1H, m, 4-H_AH_B), 2.04-2.36 (7H, m, 4-H_AH_B, 9-H₂, 6-H₂, 15-H₂), 2.43 (1H, br t, *J* 6.2, 8-H), 3.68-3.79 (2H, m, 16-H₂), 4.48-4.49 (1H, m, H-3) and 4.51-4.55 (1H, m, 10-H) [13-H obscured by HOD]; δ_C (100 MHz; D₂O) 18.2 (CH₂, 5-C), 23.9 (CH₂, 14-C), 24.2 (CH₂, 15-C), 26.0 (CH₂, 6-C), 26.8
20 (CH₂, 4-C), 28.0 (CH₂, 7-C), 30.3 (CH₂, 9-C), 38.2 (CH, 8-C), 47.1 (CH₂, 16-C), 50.6 (CH, 10-C), 50.9 (CH, 3-C), 59.5 (CH, 13-C), 116.25 (q, *J* 291.7, CF₃), 162.8 (q, *J* 35.2, CF₃CO₂H), 169.2 (quat., 2-C), 174.0 (quat., 10-CO), and 179.8 (quat., 8-CO); *m/z* (FAB+) 356.1811 [MH(free base)]⁺. C₁₆H₂₆N₃O₆ requires 356.1821].

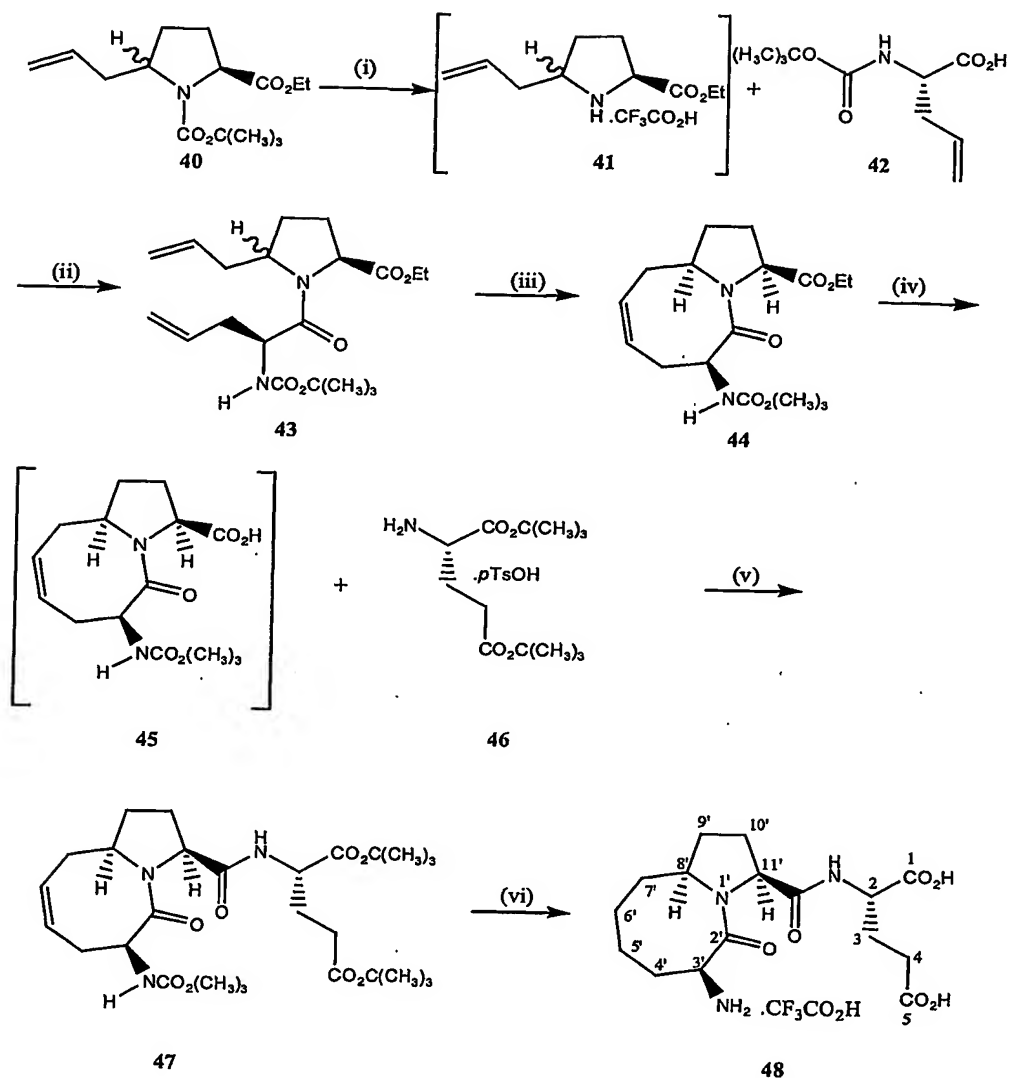
25

Example 4: Synthesis of (2*S*, 3'*S*, 8'*R*,)-2-[[*(3'-Amino-1'-aza-2'-oxo-bicyclo[6.3.0]undecyl)11'-carbonyl*amino]-1,5-pentandioic acid trifluoroacetate salt

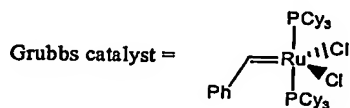
5



Scheme 1 Reagents, conditions and yields: (i) EtOH, SOCl₂, 0 °C to RT, overnight; (ii) DMAP, Boc₂O, CH₃CN, RT, 18 h (85% over 2 steps); (iii) LiEt₃BH, THF, -78 °C, 1 h, then 30% H₂O₂, 0 °C, 0.5 h; (iv) BF₃·OEt₂, allyltributylstannane, CH₂Cl₂, -78 °C, 2 h (54%, over 2 steps).



Scheme 2 Reagents, conditions and yields: (i) $\text{CF}_3\text{CO}_2\text{H}$, CH_2Cl_2 , RT, 4 h; (ii) DCC, Et_3N , CH_2Cl_2 , 0 °C to RT, overnight (55%, 2 steps); (iii) Grubbs's catalyst, CH_2Cl_2 , 45 °C, 24 h then DMSO, RT, overnight (74%); (iv) 1M aq. NaOH, H_2O , dioxane, RT, 23 h; (v) BoP-Cl, Et_3N , CH_2Cl_2 , 0 °C to RT, 21 h (70%, 2 steps); (vi) PtO_2 , H_2 , THF, RT, overnight, then $\text{CF}_3\text{CO}_2\text{H}$, CH_2Cl_2 , RT, 5 h (60%, 2 steps).



(S)-Ethyl *N*-*tert*-butoxycarbonylpyroglutamate **38**

(*S*)-Pyroglutamic acid **9** (3.0 g, 15.5 mmol) was suspended in ethanol
5 (40 cm³) and cooled to 0 °C under a nitrogen atmosphere. Thionyl chloride
(2.03 g, 17.0 mmol) was added dropwise and the solution was allowed to warm
to room temperature overnight. The solution was cooled to 0 °C, neutralised
with saturated aqueous sodium hydrogen carbonate and extracted with
chloroform. The combined organic layers were pooled, dried (Na₂SO₄), filtered
10 and the solvent removed to give the crude *ester* **37** (2.53 g, 104%) which was
used without further purification. To a solution of *ester* **37** (2.53 g) and
dimethylaminopyridine (0.19 g, 1.55 mmol) in acetonitrile (30 cm³) was added
di-*tert*-butyl dicarbonate (3.71 g, 17.05 mmol). The resultant solution was
stirred at room temperature for 18 h, concentrated *in vacuo* and purified by
15 chromatography (SiO₂, 3:1, 2:1, hexanes-ethyl acetate) to give a yellow solid
that was recrystallised from hexanes to afford *carbamate* **38** (3.35 g, 85%, in 2
steps) as a white solid: mp 53-54 °C (lit. 53-54 °C); [α]_D -39.8 (*c* 0.5 in MeOH)
[lit. -46.3 (*c* 1.5 in MeOH)]; δ_{H} (300 MHz; CDCl₃; Me₄Si) 1.26 (3H, t, *J* 7.1,
OCH₂CH₃), 1.48 [9H, s, C(CH₃)₃], 1.96-2.06 (1H, m), 2.24-2.68 (3H, m), 4.22
20 (2H, q, *J* 7.2, OCH₂CH₃), and 4.58 (1H, dd, *J* 9.4 and 3.0, 5-H); δ_{C} (75 MHz;
CDCl₃) 14.2 (CH₃, OCH₂CH₃), 21.5 (CH₂, 4-C), 27.9 [CH₃, C(CH₃)₃], 31.2
(CH₂, 3-C), 58.9 (CH, 5-C), 61.7 (CH₂, OCH₂CH₃), 83.5 [quat., C(CH₃)₃],
149.3 (quat., NCO₂), 171.3 (quat., 5-CO) and 173.2 (quat., 2-C).

25 *N*-*tert*-Butoxycarbonyl-(*S*)-allylglycine-5-allyl-*L*-proline ethyl ester **43**

To a stirred solution of *carbamate* **38** (1.0 g, 3.89 mmol) in dry
tetrahydrofuran (40 cm³) was added a 1 mol L⁻¹ tetrahydrofuran solution of
lithium triethylborohydride (4.66 cm³, 4.66 mmol) at -78 °C under at
atmosphere of nitrogen. The solution was stirred for 1 h, saturated aqueous
30 sodium hydrogen carbonate (10 cm³) was added and the cooling bath removed.

The temperature was allowed to reach 0 °C then 30% aqueous hydrogen peroxide (35 drops) was added and stirring was continued at 0 °C for 50 min. The aqueous layer was extracted with ether, the combined organic extracts were washed with brine, dried (MgSO₄), filtered and the solvent removed to give
5 *alcohol 39* (1.301 g). Crude *alcohol 39* (0.363 g, *ca.* 1.40 mmol) was dissolved in dry dichloromethane (10 cm³), allyltributylstannane (0.87 cm³, 2.80 mmol) was added and the solution cooled to -78 °C under a nitrogen atmosphere. Boron trifluoride etherate (0.36 cm³, 2.80 mmol) was added dropwise, and the solution was stirred at -78 °C for 2 h, then saturated aqueous sodium hydrogen
10 carbonate (10 cm³) was added and the reaction mixture warmed to room temperature. The aqueous layer was extracted with dichloromethane and the pooled organic extracts dried (Na₂SO₄) and filtered. Removal of the solvent *in vacuo* afforded an oil (1.28 g) which was purified by chromatography (SiO₂, 16:1, 10:1, 8:1, 6:1, 4:1, 3:1, hexanes-ethyl acetate) to afford *alkene 40* [0.217
15 g, 54%, in 2 steps (74% brsm)] as a colourless oil. *Alkene 40* was shown to be a 66:33 mixture of C(1)/C(5) *cis:trans* isomers together with minor component(s). Due to the complex nature of the NMR spectrum this compound was not characterized and used as such for the next step.

Trifluoroacetic acid (2 cm³) was added to a solution of *alkene 40* (0.22
20 g, 0.77 mmol) in dichloromethane (4 cm³) and the solution was stirred at room temperature for 4 h, then the volatiles removed *in vacuo* to yield the *trifluoroacetate salt 41* as an oil. Half of this material (0.383 mmol) was dissolved in dichloromethane (7 cm³), *N-tert*-butoxycarbonyl-(*S*)-allylglycine **42** (0.07 g, 0.32 mmol) and triethylamine (0.042 g, 0.42 mmol) were added and
25 the solution cooled to 0 °C. *N, N'*-Dicyclohexylcarbodiimide (0.065 g, 0.316 mmol) was added, the mixture stirred overnight, then filtered through Celite™ to remove dicyclohexyl urea. The filtrate was subsequently washed with saturated aqueous sodium hydrogen carbonate, 2 M aqueous hydrochloric acid, dried (MgSO₄), filtered and the solvent removed to yield an oil (0.113 g) which
30 was purified by chromatography (SiO₂, 4:1, hexane-ethyl acetate) to afford

diene **43** (0.064 g, 55%, 2 steps) as a colourless oil. Diene **43** was an inseparable mixture of diastereomers [C2/C5, *cis/trans*, 77:23]: δ_H (400 MHz; CDCl₃; Me₄Si) 1.21-1.26 (3H, m, OCH₂CH₃), 1.37 [6.93H, s, C(CH₃)₃], 1.41 [2.07H, s, C(CH₃)₃], 1.64-1.97 (4H, m, Pro β -H₂ and Pro γ -H₂), 2.0-2.52 [3.77H, m, 2 x CH₂(allyl)], 2.75-2.95 [0.23H, m, CH_ACH_B(allyl)], 4.07-4.21 (2.23H, m, OCH₂CH₃ and Pro α -H*), 4.32-4.48 (2.77H, Pro δ -H, Gly α -H and Pro α -H), 4.97-5.26 (5H, m, 2 x =CH₂, N-H) and 5.64-5.84 [2H, m, 2 x C(H)=CH₂]; δ_C (100 MHz; CDCl₃) 13.9* (CH₃, OCH₂CH₃), 14.1 (CH₃, OCH₂CH₃), 26.8 (CH₂, Pro β -C), 28.3 [CH₃, C(CH₃)₃], 29.0* (CH₂, Pro γ -C), 29.5 (CH₂, Pro γ -C), 37.7* [CH₂, CH₂(allyl)Pro], 37.8 [CH₂, CH₂(allyl)Gly], 38.4* [CH₂, CH₂(allyl)Gly], 39.4* [CH₂, CH₂(allyl)Pro], 50.9 (CH, Gly α -C), 51.9* (CH, Gly α -C), 58.2 (CH, Pro α -C), 58.5* (CH, Pro α -C), 59.5 (CH, Pro δ -C), 59.8* (CH, Pro δ -C), 61.0 (CH₂, OCH₂CH₃), 61.9* (CH₂, OCH₂CH₃), 79.4* [quat., C(CH₃)₃], 79.7 [quat., C(CH₃)₃], 117.2* (CH₂, =CH₂), 118.3 (CH₂, =CH₂), 118.35 (CH₂, =CH₂), 118.7* (CH₂, =CH₂), 132.7* (CH, C(H)=CH₂), 133.2 [CH, C(H)=CH₂], 134.0 [CH, C(H)=CH₂], 134.7* [CH, C(H)=CH₂], 154.7* (quat., NCO₂), 155.3 (quat., NCO₂), 170.5* (quat., CO), 171.4 (quat., CO), 171.6* (quat., CO) and 172.1 (quat., CO); *m/z* (FAB+) 381.23896 (MH⁺. C₂₀H₃₃N₂O₅ requires 381.23895).

20 (8*R*, 3*S*, 11*S*) 1-Aza-3-(*tert*-butyloxycarbonylamino)-11-ethoxycarbonyl-2-oxobicyclo[6.3.0]undec-5-ene **44**

To a degassed solution of diene **43** (0.064 g, 0.170 mmol) in dry dichloromethane (43 cm³) was added bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs catalyst) (0.014 g, 0.0170 mmol) under a nitrogen atmosphere and the resultant purple solution heated at reflux for 24 h. The orange/brown solution was cooled to room temperature, dimethyl sulphoxide (0.160 cm³, 2.26 mmol) was added and the solution stirred overnight. The solvent was removed *in vacuo* and the

residue purified by chromatography (SiO₂, 2:1, 1:1, hexanes-ethyl acetate,) to give *alkene 44* (0.044 g, 74%) as a colourless oil. *Alkene 44* existed exclusively as the *trans* C(O)-NPro conformer: $[\alpha]_D -93.2$ (*c* 0.29 in CH₂Cl₂); δ_H (400 MHz; CDCl₃; Me₄Si) 1.25 (3H, t, *J* 7.1, OCH₂CH₃), 1.42 [9H, s, C(CH₃)₃], 1.89-1.98 (2H, m, 10-*H_AH_B* and 9-*H_AH_B*), 2.01-2.09 (1H, m, 9-*H_AH_B*), 2.11-2.16 (1H, m, 10-*H_AH_B*), 2.24-2.33 (1H, m, 4-*H_AH_B*), 2.42 (1H, dq, *J* 15.2 and 3.4, 7-*H_AH_B*), 2.70-2.80 (2H, m, 4-*H_AH_B* and 7-*H_AH_B*), 4.15 (3H, q, *J* 7.1, OCH₂CH₃ and 8-H obscured), 4.47 (1H, dd, *J* 8.4 and 2.8, 11-H), 4.84 (3H, br q, *J* 7.8, 3-H), 5.59 (1H, d, *J* 7.3, N-H), 5.67-5.73 (1H, m, 6-H) and 5.77-5.83 (1H, m, 5-H); δ_C (100 MHz; CDCl₃) 14.0 (CH₃, OCH₂CH₃), 27.1 (CH₂, 10-C), 28.2 [CH₃, C(CH₃)₃], 32.77 (CH₂, 7-C or 9-C), 32.84 (CH₂, 9-C or 7-C), 35.2 (CH₂, 4-C), 51.7 (CH, 3-C), 58.6 (CH, 8-C), 60.2 (CH, 11-C), 60.8 (CH₂, OCH₂CH₃), 79.4 [quat., C(CH₃)₃], 125.7 (CH, 6-C), 129.1 (CH, 5-C), 155.1 (quat., NCO₂), 170.9 (quat., 11-CO) and 171.7 (quat., 2-C); *m/z* (EI⁺) 352.2001 (M⁺. C₁₈H₂₈N₂O₅ requires 352.1998).

(2*S*, 3'*S*, 8'*R*, 11'*S*)-Di-*tert*-butyl 2-[[1'-*aza*-3'-(*tert*-butyloxycarbonylamino)-2'-oxobicyclo[6.3.0]-undec-5'-ene]-11-carbonyl]amino}-1,5-pentandioate **47**

1M Aqueous sodium hydroxide (0.63 cm³, 0.63 mmol) was added to a solution of ester **44** in dioxane (1.3 cm³) and the opaque solution stirred for 23 h at room temperature. The reaction mixture was diluted with water, extracted with dichloromethane and the aqueous layer acidified with solid citric acid and extracted with dichloromethane. The combined organic layers were dried (Na₂SO₄), filtered and the solvent removed to yield *acid 45* (0.040 g). Hydrochloride **46** (0.048 g, 0.1625 mmol) was added to a solution of **45** and the solution then cooled to 0 °C. Triethylamine (0.033 cm³, 0.325 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoP-Cl) (0.041 g, 0.163 mmol) were added and the mixture stirred for 21 h. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate, 2 M aqueous hydrochloric acid, dried (Na₂SO₄), filtered and the solvent removed to yield an oil (0.078 g) which

was purified by chromatography (SiO₂, 1:1, hexane-ethyl acetate) to afford amide 47 (0.050 g, 70%, in 2 steps) as a colourless oil. Amide 47 existed exclusively as the *trans* C(O)-NPro conformer: $[\alpha]_D -79.4$ (c 0.47 in CH₂Cl₂); δ_H (400 MHz; CDCl₃; Me₄Si) 1.39-1.50 [27H, m, 3 x C(CH₃)₃], 1.78-1.91 (3H, m, 10'-H_AH_B, 3-H_AH_B and 9'-H_AH_B), 2.0-2.33 (7H, m, 10'-H_AH_B, 3-H_AH_B, 4-H₂, 9'-H_AH_B, 7'-H_AH_B and 4'-H_AH_B), 2.80-2.95 (2H, br m, 7'-H_AH_B and 4'-H_AH_B), 4.15 (1H, br q, *J* 7.1, 8'-H), 4.42 (1H, ddd, *J* 7.8, 7.8 and 5.2, 2-H), 4.66 (1H, d, *J* 7.5, 11'-H), 4.97 (1H, br q, *J* 7.4, 3'-H), 5.57 (1H, d, *J* 7.8, N-H), 5.61-5.67 (1H, m, 6'-H), 5.81 (1H, br t, *J* 7.8, 5'-H) and 7.47 (1H, d, *J* 8.0, N-H); δ_C (100 MHz; CDCl₃) 26.1 (CH₂, 10'-C), 27.9 [CH₃, C(CH₃)₃], 27.95 [CH₃, C(CH₃)₃], 28.2 [CH₃, C(CH₃)₃], 28.1, (CH₂, 3-C), 31.3 (CH₂, 4-C), 32.0 (CH₂, 7'-C), 33.2 (CH₂, 9'-C), 35.7 (CH₂, 4'-C), 51.0 (CH, 3'-C), 51.9 (CH, 2-C), 58.6 (CH, 8'-C), 60.8 (CH, 11'-C), 79.6 [quat., C(CH₃)₃], 80.4 [quat., C(CH₃)₃], 81.9 [quat., C(CH₃)₃], 124.9 (CH, 6'-C), 130.4 (CH, 5'-C), 155.0 (quat., NCO₂), 169.9 (quat., 1-C), 170.5 (quat., 11'-CO), 171.8 (quat., 2'-C or 5-C) and 171.9 (quat., 2'-C or 5-C); *m/z* (FAB+) 566.3454 [MH⁺. C₂₉H₄₈N₃O₈ requires 566.3441].

(2*S*, 3'*S*, 8'*R*, 11'*S*) 2-{[(3'-Amino-1'-aza-2'-oxobicyclo[6.3.0]-undecyl)-11'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 48

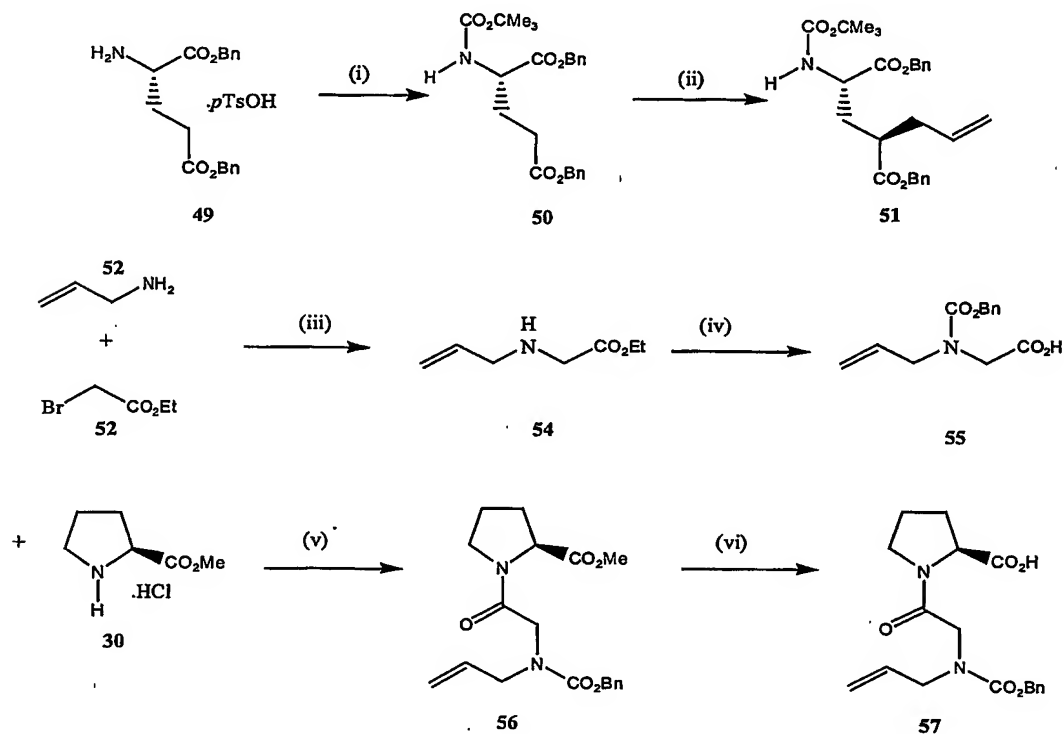
PtO₂ (0.00184 g, 0.0081 mmol) was added to a stirred solution of amide 47 (0.046 g, 0.081 mmol) in tetrahydrofuran (4 cm³) under a nitrogen atmosphere. The mixture was hydrogenated (1 atm. of hydrogen) overnight, filtered through Celite™, and the solvent removed in vacuo. The residue was dissolved in dichloromethane (5 cm³), trifluoroacetic acid (3 cm³) added and the solution stirred for 5 h at room temperature. Removal of the volatiles in vacuo, purification by RP HPLC [10% acetonitrile:90% water (containing 0.05% trifluoroacetic acid)] and trituration from ether/toluene gave 13 (0.0228 g, 60%, 2 steps) as a hygroscopic off-white solid. 48 existed exclusively as the *trans*

C(O)-NPro conformer: mp 50-120 °C; $[\alpha]_D$ -27.9 (c 0.33 in MeOH); δ_H (400 MHz; D₂O) 1.37-1.85 (8H, m, 5'-H₂, 6'-H₂, 4'-H₂ and 9'-H₂), 1.98-2.28 (5H, m, 3-H₂, 7'-H₂ and 10'-H_AH_B), 2.36 (1H, dt, J 12.4 and 7.2, 10'-H_AH_B), 2.57 (2H, t, J 7.6, 4-H₂), 4.23 (1H, br t, J 8.2, 8'-H) and 4.41 (3H, m, 2-H, 11'-H, 3'-H);

5 δ_C (100 MHz; D₂O) 21.6 (CH₂, 5'-C or 6'-C), 24.2 (CH₂, 5'-C or 6'-C), 25.5 (CH₂, 3-C), 27.1 (CH₂, 10'-C), 29.7 (CH₂, 4-C), 31.5 (CH₂, 9'-C), 32.8 (CH₂, 7'-C), 35.0 (CH₂, 4'-C), 51.2 (CH, 3'-C), 51.9 (CH, 2-C), 59.9 (CH, 8'-C), 61.4 (CH, 11'-C), 116.2 (quat., q, J 291, CF₃), 162.7 (quat., q, J 35.2, CF₃CO₂H), 168.5 (quat., 2'-C), 173.7 (quat., 11'-CO), 174.7 (quat., 1-C) and 176.8 (quat.,

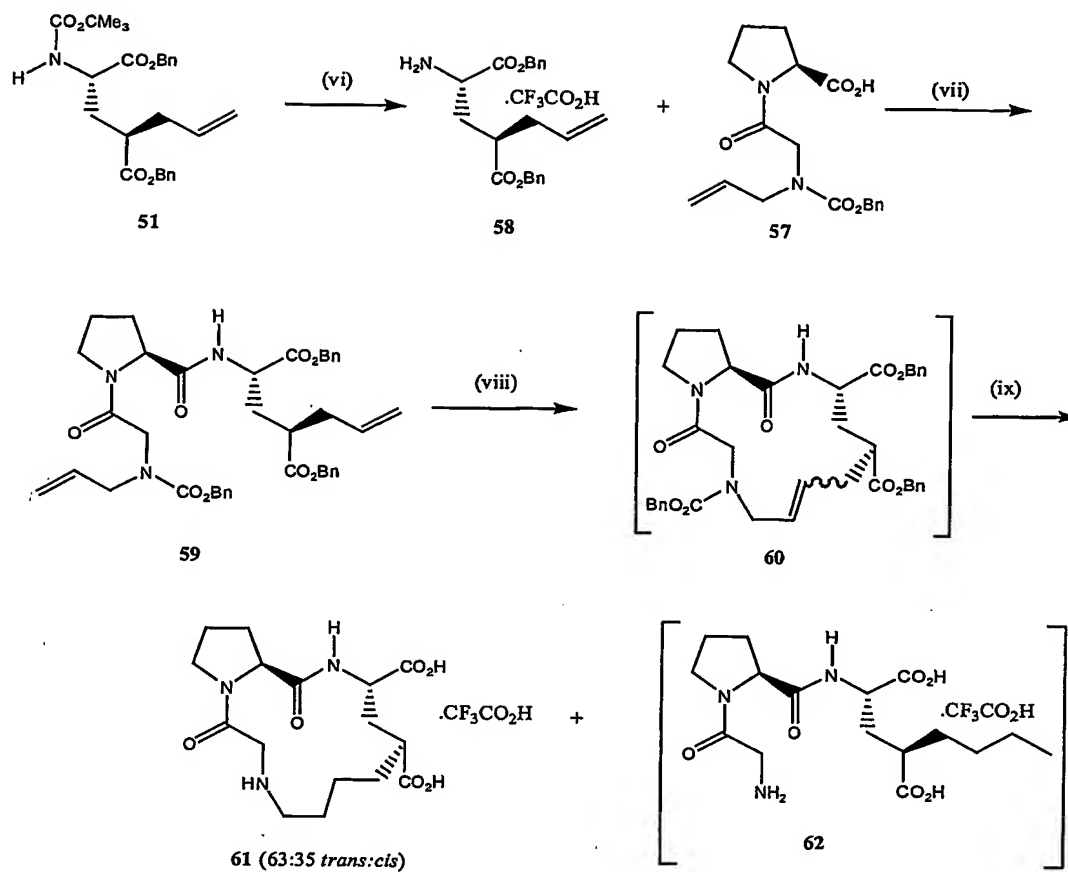
10 5-C); m/z (FAB+) 356.1816 [MH(free base)]⁺. C₁₆H₂₆N₃O₆ requires 356.1822].

Example 5: Synthesis of (9*R*, 11*S*, 14*S*)-1,4,12-triaza-9,11-carboxy-2,13-dioxobicyclo[14.3.0]heptadecane trifluoroacetate

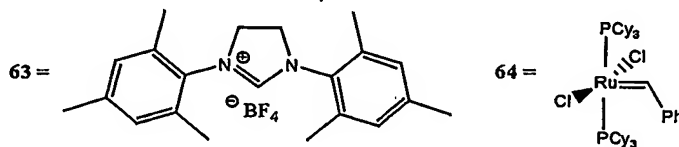


Scheme 1 Reagents, conditions and yields: (i) Boc_2O , Et_3N , CH_2Cl_2 , 0 °C to RT, 19 h (97%); (ii) LiHMDS , THF, -78 °C, 40 mins, then allyl bromide, -78 °C, 1.5 h (73%); (iii) THF, 0 °C, 2.5 h (47%); (iv) BnOCOC l, Et_3N , CH_2Cl_2 , 0 °C, 2 h then 1M aq. NaOH , dioxane, methanol, RT, 2 h (52% over 3 steps); (v) EDCI , Et_3N , CH_2Cl_2 , 0 °C to RT, 19 h (66%); (vi) 1M aq. NaOH , dioxane, RT, 20 h (55%).

5



Scheme 2 Reagents, conditions and yields: (vi) TFA, CH_2Cl_2 , 0 °C, 2 h (vii) EtOCOCl , Et_3N , CH_2Cl_2 , 0 °C then 58, overnight (94%); (viii) KO^tBu , THF, imidazolium ligand 63, 2 min then Grubbs's catalyst 64, benzene, 80 °C, 35 min, then 59, benzene, 40 °C, 65 h; (ix) 10% Pd/C, H_2 , $\text{MeOH}/\text{H}_2\text{O}$ (4:1), RT, 17 h (58% from 59).



N-tert-Butyloxycarbonyl-L-glutamic acid dibenzyl ester 50

Triethylamine (2.25 cm³, 2.2 mmol) was added to a stirred solution of L-glutamic acid dibenzyl ester *p*-toluenesulphonate **49** (4.0 g, 8.01 mmol) in dichloromethane (80 cm³) at 0 °C. Di-*tert*-butyldicarbonate (2.1 g, 9.61 mmol) was then added and the solution stirred for 19 h. The solvent was removed *in vacuo*, the residue dissolved in the minimum amount of dichloromethane and washed sequentially with 2M aqueous hydrochloric acid, saturated aqueous sodium hydrogen carbonate and dried (MgSO₄). Removal of the solvent yielded a white solid (3.42 g) which was purified by crystallisation from ether/hexanes to give *carbamate 50* (3.321 g, 97%) as a white solid: mp 73-76 °C; δ_{H} (400 MHz; CDCl₃; Me₄Si) 1.46 [9H, s, C(CH₃)₃], 1.96-2.07 (1H, m), 2.22-2.27 (1H, m), 2.38-2.52 (2H, m), 4.40 (1H, br d, *J* 3.6, Glu α -H), 5.12-5.19 (5H, m, 2 x OCH₂Ph, Glu α -NH) and 7.29-7.39 (10H, m, 2 x Ph) δ_{C} (100 MHz; CDCl₃) 27.6 (CH₂, Glu β -C), 28.1 [CH₃, C(CH₃)₃], 30.1 (CH₂, Glu γ -C), 52.8 (CH, Glu α -C), 66.4 (CH₂, OCH₂Ph), 67.1 (CH₂, OCH₂Ph), 79.7 [quat., C(CH₃)₃], 128.1, 128.2, 128.3, 128.5, 128.51, (CH, Ph), 135.2, 135.7 (quat., Ph), 155.3 (quat., NCO₂) and 172.0, 172.4 (quat., CO).

N-tert-Butyloxycarbonyl-L- γ (R)-allylglutamic acid dibenzyl ester 51

A tetrahydrofuran solution of lithium hexamethyldisilazide (1 mol L⁻¹, 10.3 cm³, 10.3 mmol) was added dropwise to a solution of *carbamate 50* (2.0 g, 4.68 mmol) in dry tetrahydrofuran (20 cm³) at -78 °C under an atmosphere of nitrogen. The resultant yellow solution was stirred at this temperature for 40 min and allyl bromide (1.31 cm³, 14.0 mmol) was added. The solution was stirred for 1.5 h at -78 °C and quenched by the addition of saturated aqueous ammonium chloride (20 cm³). The cooling bath was removed and the mixture allowed to warm to room temperature over 1.5 h. A white solid was removed by filtration, washed with ethyl acetate and the filtrate concentrated *in vacuo* to yield an orange oil (2.312 g) which was purified by chromatography (silica gel,

6:1, hexane:ethyl acetate, then 5:1) to give *alkene 51* (1.589 g, 73%) as a colourless oil: $[\alpha]_D +19.4$ (c 0.232 in CH_2Cl_2); δ_H (300 MHz; CDCl_3 ; Me_4Si) 1.43 [9H, s, $\text{C}(\text{CH}_3)_3$], 2.06 (2H, m, allyl- CH_2), 2.30-2.44 (2H, m, Glu β -H₂), 2.64 (1H, p, J 6.7, Glu γ -H), 4.41 (1H, br d, J 7.6, Glu α -H), 5.01-5.71 (7H, m, 2 x OCH_2Ph , $=\text{CH}_2$, Glu α -NH), 5.60-5.74 (1H, m, $\text{C}(\text{H})=\text{CH}_2$) and 7.31-7.35 (10H, m, 2 x Ph); δ_C (75 MHz; CDCl_3) 28.2 [CH_3 , $\text{C}(\text{CH}_3)_3$], 33.4 (CH_2 , allyl- CH_2), 36.2 (CH_2 , Glu β -C), 41.7, (CH, Glu γ -C), 52.2 (CH, Glu α -C), 66.4 (CH_2 , OCH_2Ph), 67.0 (CH_2 , OCH_2Ph), 80.0 [quat., $\text{C}(\text{CH}_3)_3$], 117.6 (CH_2 , $=\text{CH}_2$), 128.16, 128.18, 128.3, 128.44, 128.47, (CH, Ph), 134.1 (CH, $\text{C}(\text{H})=\text{CH}_2$), 135.2, 135.7 (quat., Ph), 155.3 (quat., NCO_2), 172.1 (quat., Glu α -CO) and 174.6 (quat., Glu γ -CO); m/z (FAB+) 468.2382 (MH^+ . $\text{C}_{27}\text{H}_{34}\text{NO}_6$ requires 468.2386).

N-Allylglycine ethyl ester 54

A solution of ethyl bromoacetate **52** (3 g, 18.0 mmol) in dry tetrahydrofuran (20 cm^3) was added dropwise to an ice-cold solution of allylamine **53** (2.05 g, 36 mmol) in dry tetrahydrofuran (20 cm^3) over 3 min. The solution was stirred at 0 °C for 2.5 h (a white precipitate was observed after 2 h), concentrated *in vacuo* and suspended in diethyl ether. The white suspension was filtered through Celite™ and the solvent removed to yield the crude product which was purified by chromatography (silica gel, 1:1, hexane:ethyl acetate) to give *alkene 54* (1.209 g, 47%) as a light yellow oil: δ_H (300 MHz; CDCl_3 ; Me_4Si) 1.17 (3H, t, J 7.1, OCH_2CH_3), 1.70 (1H, br s, N-H), 3.16 [2H, d, J 5.9, $\text{CH}_2(\text{allyl})$], 3.28 (2H, s, CH_2Gly), 4.08 (2H, q, J 7.1, OCH_2CH_3), 4.98-5.12 (2H, m, $=\text{CH}_2$) and 5.70-5.85 (1H, m, $\text{C}(\text{H})=\text{CH}_2$); δ_C (75 MHz; CDCl_3) 13.9 (CH_3 , OCH_2CH_3), 49.7 (CH_2), 51.6 (CH_2), 60.4 (CH_2 , OCH_2CH_3), 116.2 (CH_2 , $=\text{CH}_2$), 135.9 (CH, $\text{C}(\text{H})=\text{CH}_2$) and 172.2 (quat., CO).

N-Allyl-N-benzyloxycarbonylglycine 55

N-Allylglycine ethyl ester **54** was prepared as described above using ethyl bromoacetate (9 g, 53.9 mmol) and allylamine (6.15 g, 107.7 mmol). The crude product (7.054 g) that contained ~30% of *N*-allyl-*N*-bis(2-carboethoxyethyl)amine as judged by NMR, was dissolved in dichloromethane (50 cm³), cooled to 0 °C and triethylamine (6.53 g, 64.09 mmol) added. Benzylchloroformate (9.25 g, 54.2 mmol) was added dropwise over 10 min resulting in a white precipitate. After 2 h the mixture was stored overnight at 0 °C, filtered and the filtrate concentrated *in vacuo*. The residue was subsequently suspended in dichloromethane, washed with 2M aqueous hydrochloric acid, dried (MgSO₄) and the solvent removed to reveal an oil (13.65 g) containing the desired carbamate (70%) and benzyl alcohol (30%) as judged by NMR. This mixture was dissolved in dioxane/methanol (8:3, 110 cm³), 1M aqueous sodium hydroxide (40 cm³, 40 mmol) added and the solution stirred for 2 h at room temperature. Brine (50 cm³) was added and the aqueous layer extracted with diethyl ether. The aqueous layer was acidified with 32% hydrochloric acid and extracted with ethyl acetate. The combined organic layers were dried (MgSO₄), concentrated *in vacuo*, and the residue purified by chromatography (silica gel, hexane:ethyl acetate:acetic acid, 3:1:0.4) to give *acid* **7** (7.05 g, 52%, 3 steps) as a colourless syrup. Acid **55** existed as a 1:1 mixture of carbamate rotamers: δ_H (400 MHz; CDCl₃; Me₄Si) 4.02-4.08 [4H, m, CH₂(allyl), CH₂(Gly)], 5.17-5.24 (4H, m, OCH₂Ph, =CH₂), 5.77-5.84 (1H, m, C(H)=CH₂), 7.32-7.39(m, 5H, Ph) and 8.45 (1H, br s, OH); δ_C (100 MHz; CDCl₃) 47.1, 47.7 [CH₂, CH₂(Gly)], 50.5, 50.7 [CH₂, CH₂(allyl)], 67.6, 67.7 (CH₂, OCH₂Ph), 117.8, 118.3 (CH₂, =CH₂), 127.7, 127.97, 128.01, 128.4, (CH, Ph), 132.7, 132.8 (CH, C(H)=CH₂) 136.1 (quat., Ph), 155.8, 156.5, (quat., NCO₂) and 174.7, 174.9 (quat., CO).

N-Allyl-N-benzyloxycarbonylglycyl-L-proline methyl ester 56

1-(3-Dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (EDCI) (0.453 g, 2.36 mmol) was added to a solution of proline methyl ester hydrochloride **30** (0.356 g, 2.15 mmol), acid **55** (0.590 mmol, 2.35 mmol) and triethylamine (0.481 g, 4.73 mmol) in dichloromethane (20 cm³) at 0 °C. The resultant solution was stirred for 19 h, washed with 2M aqueous hydrochloric acid and saturated aqueous sodium hydrogen carbonate, dried (MgSO₄) and the solvent removed to yield an oil which was purified by chromatography (silica gel, hexanes:ethyl acetate, 2:1, 1:1) to afford *protected dipeptide 56* (0.508 g, 66%) as a colourless oil. Dipeptide **56** was shown to be a 76:24 *trans:cis* mixture of conformers by ¹³C NMR analysis (the ratio was estimated by integration of signals at δ 22.0 and 24.5, 24.7, assigned to the Proγ-C atoms of the minor and major conformers respectively). In addition, restricted rotation about the GlyN-CO carbamate bond was also observed resulting in a further 1:1 mixture of conformers: [α]_D -54.6 (c 1.17 in CH₂Cl₂); δ_H (400 MHz; CDCl₃; Me₄Si) 1.81-2.14 (4H, m, Proβ-H₂, Proγ-H₂), 3.29-3.58 (2H, m, Proδ-H₂), 3.64, 3.66, 3.69* (3H, s, OCH₃), 3.84-4.20 [4H, m, CH₂(allyl), Glyα-H₂], 4.40-4.48 (1H, m, Proα-H), 5.07-5.17 (4H, m, OCH₂Ph, =CH₂), 5.69-5.81 (1H, m, C(H)=CH₂) and 7.24-7.30 (m, 5H, Ph); δ_C (100 MHz; CDCl₃) 22.0* (CH₂, Proγ-C), 24.62, 24.73 (CH₂, Proγ-C), 28.66, 28.73 (CH₂, Proβ-C), 31.1, * 31.3* (CH₂, Proβ-C), 45.93, 45.98 (CH₂, Proδ-C), 46.5* (CH₂, Proδ-C), 47.5, 48.0 (CH₂, Glyα-C), 50.0, 50.6 [CH₂, CH₂(allyl)], 50.0, * 50.6* [CH₂, CH₂(allyl)], 52.0, (CH₃, OCH₃), 52.31, * 52.5* (CH₃, OCH₃), 58.4, * 58.5* (CH, Proα-C), 58.8 (CH, Proα-C), 67.2, 67.3 (CH₂, OCH₂Ph), 117.0, 117.5 (CH₂, =CH₂), 127.5, 127.7, 127.8, 128.3, (CH, Ph), 133.2, 133.4 (CH, C(H)=CH₂) 136.4, 136.5 (quat., Ph), 155.8, 156.3, (quat., NCO₂), 167.1 (quat., Gly-CO), 167.4, * 167.5* (quat., Gly-CO), 172.0, * 172.2* (quat., Pro-CO) and 172.3, 172.4 (quat., Pro-CO); *m/z* (EI⁺) 360.1676 (M⁺. C₁₉H₂₄N₂O₅ requires 360.1685).

N-Allyl-N-benzyloxycarbonylglycyl-L-proline 57

To a solution of protected dipeptide **56** (0.474 g, 1.31 mmol) in dioxane (13 cm³) was added 1M aqueous sodium hydroxide (6.71 cm³, 6.71 mmol) and the mixture stirred at room temperature for 20 h. Water (10 cm³) was added and the mixture extracted with dichloromethane. The aqueous layer was acidified with 10% HCl and the product extracted with dichloromethane. The organic layers were pooled, dried (MgSO₄) and the solvent removed to afford an oil (0.456 g) contaminated with 2-hydroxy-1,4-dioxane. Subsequent purification by chromatography (silica gel, hexanes:ethyl acetate, 1:1, 1:2, 1:3,) gave *acid 57* (0.250 g, 55%) as a colourless oil: Acid **57** was shown to be a 86:14 *trans:cis* mixture of conformers by ¹³C NMR analysis (the ratio was estimated by integration of signals at δ 22.1 and 24.6, 24.7, assigned to the Pro γ -C atoms of the minor and major conformers respectively). In addition, restricted rotation about the GlyN-CO carbamate bond was also observed resulting in a further 1:1 mixture of conformers [α]_D -117 (*c* 0.8 in CH₂Cl₂); δ_H (300 MHz; CDCl₃; Me₄Si) 1.88-2.25 (4H, m, Pro β -H₂, Pro γ -H₂), 3.30-3.69 (2H, m, Pro δ -H₂), 3.75-4.26 [4H, m, CH₂(allyl), Gly α -H₂], 4.50-4.58 (1H, m, Pro α -H), 5.10-5.22 (4H, m, OCH₂Ph, =CH₂), 5.73-5.82 (1H, m, C(H)=CH₂), 7.33-7.34 (m, 5H, Ph) and 7.84 (1H, br s, OH); δ_C (75 MHz; CDCl₃) 22.1* (CH₂, Pro γ -C), 24.6, 24.7 (CH₂, Pro γ -C), 27.9, 28.0 (CH₂, Pro β -C), 31.1, 31.3* (CH₂, Pro β -C), 46.5 (CH₂, Pro δ -C), 46.7* (CH₂, Pro δ -C), 47.5, 48.1 (CH₂, Gly α -C), 47.7, 48.2* (CH₂, Gly α -C), 50.2, 50.7 [CH₂, CH₂(allyl)], 50.5, 50.9* [CH₂, CH₂(allyl)], 58.6* (CH, Pro α -C), 59.5 (CH, Pro α -C), 67.6 (CH₂, OCH₂Ph), 117.3, 117.9 (CH₂, =CH₂), 127.6, 127.9, 128.0, 128.4, (CH, Ph), 133.0, 133.2 (CH, C(H)=CH₂) 136.3 (quat., Ph), 155.9, 156.5, (quat., NCO₂), 156.7* (quat., NCO₂), 167.8* (quat., Gly-CO), 168.9, 169.0 (quat., Gly-CO), 173.6, 173.7 (quat., Pro-CO) and 174.4* (quat., Pro-CO); *m/z* (EI+) 346.1524 (M⁺. C₁₈H₂₂N₂O₅ requires 346.1529).

N-Allyl-N-benzyloxycarbonylglycyl-L-prolyl-L-γ(R)-allylglutamic acid dibenzyl ester 59

Alkene **51** (0.359 g, 0.768 mmol) was dissolved in dichloromethane (10 cm³), cooled to 0 °C and trifluoroacetic acid (2 cm³) was added. After stirring for 2 h the volatiles were removed *in vacuo* to yield *trifluoroacetate 58* as a white solid. To an ice cold solution of acid **57** (0.288 g, 0.655 mmol) and triethylamine (0.150 cm³, 0.80 mmol) was added dropwise ethyl chloroformate (0.075 cm³, 0.786 mmol). The solution was stirred at 0 °C for 40 min then an ice-cold solution of **58** and triethylamine (0.150 cm³, 0.80 mmol) in dichloromethane (15 cm³) was added dropwise and the mixture stirred overnight. The solution was subsequently washed with 2M aqueous hydrochloric acid, saturated aqueous sodium hydrogen carbonate, dried (MgSO₄) and the solvent removed to yield an oil which was purified by chromatography (silica gel, hexane:ethyl acetate, 1:1) to give *fully protected tripeptide 59* (0.432 g, 94%) as a colourless oil. Tripeptide **59** was shown to be a 85:15 *trans:cis* mixture of conformers by ¹³C NMR analysis (the ratio was estimated by integration of signals at δ 22.1 and 24.9 assigned to the Proγ-C atoms of the minor and major conformers respectively). In addition, restricted rotation about the GlyN-CO carbamate bond was also observed resulting in a further 1:1 mixture of conformers: [α]_D -38.7 (*c* 0.73 in CH₂Cl₂); δ_H (300 MHz; CDCl₃; Me₄Si) 1.75-2.33 [8H, m, Proβ-H₂, Proγ-H₂, Gluβ-H₂, CH₂(allyl)], 2.55-2.66 (1H, m, Gluγ-H), 3.25-3.63 (m, 2H, Proδ-H₂), 3.77-4.19 [4H, m, Glyα-H₂, CH₂(allyl)], 4.32* (0.15H, d, *J* 7.7, Proα-H), 4.46-4.60 (1.85H, m, Proα-H, Gluα-H), 4.99-5.15 (10H, m, 3 x OCH₂Ph, 2 x =CH₂), 5.57-5.82 (2H, m, 2 x C(H)=CH₂) and 7.19-7.34 (16H, m, 3 x Ph, GluN-H); δ_C (75 MHz; CDCl₃) 22.2* (CH₂, Proγ-C), 24.9 (CH₂, Proγ-C), 27.2* (CH₂, Proβ-C), 27.5 (CH₂, Proβ-C), 31.8* [CH₂, CH₂(allyl)], 32.7 [CH₂, CH₂(allyl)], 35.9 (CH₂, Gluβ-C), 36.0* (CH₂, Gluβ-C), 41.2 (CH, Gluγ-C), 41.9* (CH, Gluγ-C),

46.4 (CH₂, Proδ-C), 46.9* (CH₂, Proδ-C), 47.6* (CH₂, Glyα-C), 48.3 (CH₂, Glyα-C), 48.2* (CH₂, Glyα-C), 50.4 [CH₂, CH₂(allyl)], 50.7, 50.9 (CH₂, Gluα-C), 51.2* [CH₂, CH₂(allyl)], 51.4* (CH₂, Gluα-C), 59.96* (CH, Proα-C), 60.0 (CH, Proα-C), 60.4* (CH, Proα-C), 66.3 (CH₂, OCH₂Ph), 66.4* (CH₂, OCH₂Ph), 66.5* (CH₂, OCH₂Ph), 67.0 (CH₂, OCH₂Ph), 67.1* (CH₂, OCH₂Ph), 67.35 (CH₂, OCH₂Ph), 67.4 (CH₂, OCH₂Ph), 117.0 (CH₂, =CH₂), 117.1* (CH₂, =CH₂), 117.5 (CH₂, =CH₂), 117.6 (CH₂, =CH₂), 117.8* (CH₂, =CH₂) 118.0* (CH₂, =CH₂), 127.56*, 127.63*, 127.77*, 127.85*, 128.08, 128.13, 128.17, 128.35, 128.39, 128.43 (CH, Ph), 133.3 (CH, C(H)=CH₂), 133.5* (CH, C(H)=CH₂), 134.0* (CH, C(H)=CH₂), 134.2* (CH, C(H)=CH₂), 134.3 (CH, C(H)=CH₂), 135.2* (quat., Ph), 135.3 (quat., Ph), 135.7 (quat., Ph), 136.4 (quat., Ph), 136.6* (quat., Ph), 155.9* (quat., NCO₂), 156.5, (quat., NCO₂), 168.4 (quat., Gly-CO), 170.9*, 171.0, 171.4, 171.8* (quat., Pro-CON, Gluα-CO), 174.3* (quat., Gluy-CO) and 174.4 (quat., Gluy-CO); *m/z* (FAB+) 696.3280 (MH⁺. C₄₀H₄₆N₃O₈ requires 696.3285).

(9R, 11S, 14S)-1,4,12-Triaza-9,11-carboxy-2,13-dioxobicyclo[14.3.0]-heptadecane trifluoroacetate 61

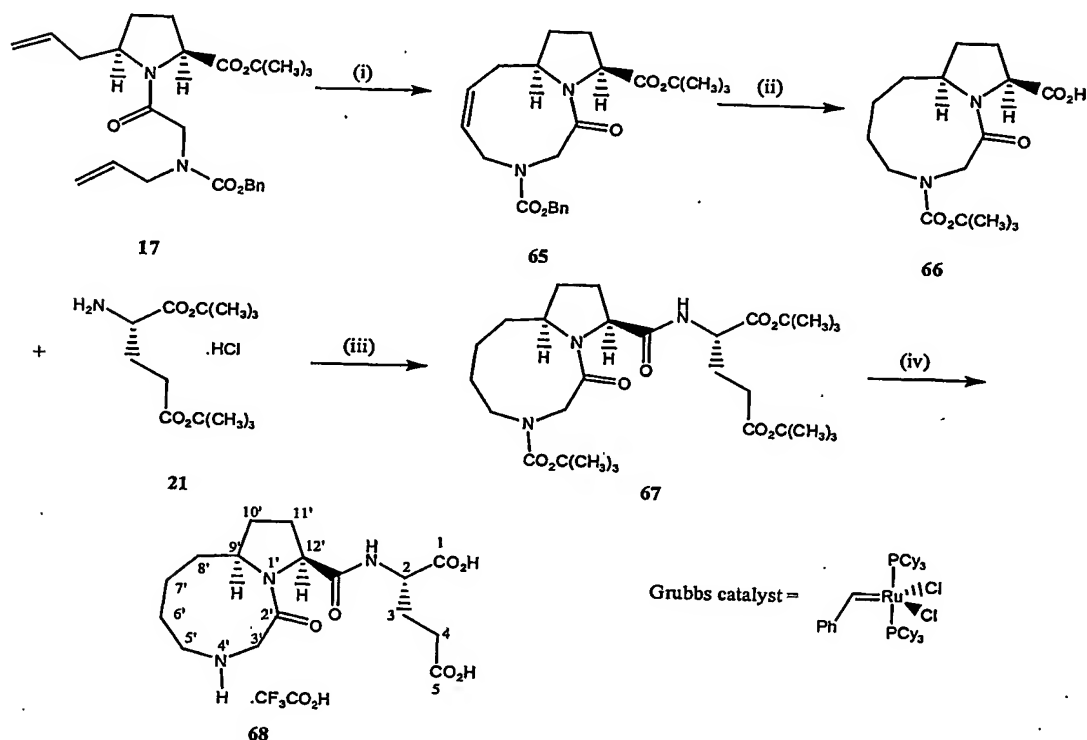
Freshly sublimed potassium *tert*-butoxide (0.009 g, 0.0792 mmol) was added to a stirred suspension of 1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazolium tetrafluoroborate³ **63** (0.031 g, 0.0792 mmol) in tetrahydrofuran (6 cm³) under an atmosphere of nitrogen. The resultant suspension was stirred for 2 min then a solution of bis(tricyclohexylphosphine)benzylideneruthenium dichloride **64** (0.054 g, 0.066 mol) in dry benzene (6 cm³) was added and the purple solution was heated at 80 °C for 35 min. The dark brown solution was cooled to room temperature and a solution of protected tripeptide **59** (0.155 g, 0.22 mmol) in dry benzene (85 cm³) added and the mixture heated at 45 °C for 65 h. The solvent was removed *in vacuo* and the residue purified by chromatography (silica gel, hexane:ethyl

acetate, 1:1, 1:2, 1:3, 1:4) to give the metathesis product **60** and unidentified product(s) (0.110 g) as a brown oil: [*m/z* (FAB+) 668.2982 (MH⁺, C₃₈H₄₂N₃O₈ requires 668.2972)]. The mixture was subsequently dissolved in methanol/water (4:1, 50 cm³) and 10 wt. % palladium on activated carbon (0.035 g, 0.033 mmol) was added under an atmosphere of nitrogen. To the reaction flask was fitted a balloon of hydrogen and the mixture stirred for 17 h at room temperature. The reaction mixture was filtered through a pad of Celite™, washed with methanol/water (4:1, 100 cm³) and concentrated *in vacuo* to yield a film (0.064 g) that was purified by RP HPLC [20% acetonitrile:80% water (containing 0.05% trifluoroacetic acid)]. Repeated evaporation from a toluene/ether mixture and drying on a freeze drier gave **61** (0.045 g, 58% from **59**) as an off white solid. **61** was shown to be a 65:35 *trans:cis* mixture of conformers by ¹H NMR analysis (the ratio was estimated by integration of signals at δ 2.99 and 3.17-3.33 assigned to the 5-H atoms of the minor and major conformers respectively): mp 120-130 °C; [α]_D -12.5 (c 0.104 in H₂O); δ_H (300 MHz; D₂O), 1.24-2.68 (13.65H, m, 5-H_AH_B, 6-H₂, 7-H₂, 8-H₂, 9-H, 10-H₂, 15-H₂, 16-H₂), 2.99* (0.35H, dt, *J* 12.9 and 7.3, 5-H_AH_B), 3.17-3.33 (1H, m, 5-H), 3.40* (0.35H, d, *J* 15.6, 3-H_AH_B), 3.50-3.73 (2H, m, 17-H₂), 3.98* (0.35H, d, *J* 15.6, 3-H_AH_B), 4.01 (0.65H, d, *J* 15.6, 3-H_AH_B), 4.08 (0.65H, d, *J* 15.8, 3-H_AH_B), 4.44 (0.65H, dd, *J* 11.6 and 4.3, 11-H), 4.51 (0.65H, dd, *J* 7.5 and 5.0, 14-H) and 4.59-4.64* (0.7H, m, 11-H, 14-H); δ_C (75 MHz; D₂O) 21.0, 21.4,* 21.7,* 22.2,* 23.6, 24.8, 25.2, 28.2, 31.2,* 31.3,* 31.6 (CH₂, 6-C, 7-C, 8-C, 10-C, 15-C, 16-C), 38.4* (CH, 9-C), 40.2 (CH, 9-C), 45.5* (CH₂, 3-C, 5-C), 46.9, 47.0 (CH₂, 17-C, 5-C), 47.5* (CH₂, 17-C), 48.2, (CH₂, 3-C), 49.6 (CH, 11-C), 50.5* (CH, 11-C), 59.9* (CH, 14-C), 61.3 (CH, 14-C), 116.2 (q, *J* 291, CF₃), 162.6 (q, *J* 35.5, CF₃CO₂H), 164.4* (quat., 2-C), 164.9 (quat., 2-C), 173.4,* 173.4* (quat., 13-C, 11-CO), 173.4, 173.4 (quat., 13-C, 11-CO), 179.3* (quat., 9-CO) and 179.8 (quat., 9-CO); *m/z* (FAB+) 356.1829 [MH(free base)⁺, C₁₆H₂₆N₃O₆ requires 356.1822]; [the minor component was tentatively assigned as glycyl-L-

prolyl-L- γ -butylglutamic acid trifluoroacetate **62** and was shown to be a 78:22 *trans:cis* mixture of conformers by ^{13}C NMR analysis (the ratio was estimated by integration of the signals at δ 51.2 and 51.8 assigned to the Glu α -C atoms of the minor and major conformers respectively): mp 155-190 °C (dec.); δ_{H} (400 MHz; D_2O), 0.90 (3H, t, J 6.5, Glu γ -CH₂CH₂CH₂CH₃), 1.33 (4H, br s, Glu γ -CH₂CH₂CH₂CH₃), 1.64 (2H, br q, J 7.0, Glu γ -CH₂CH₂CH₂CH₃), 2.03-2.17 (5H, m, Glu β -H₂, Pro γ -H₂, Pro β -H_AH_B), 2.31-2.35 (0.78H, m, Pro β -H_AH_B), 2.57 (1H, p, J 6.9, Glu γ -H), 3.59-3.75 (2.18H, m, Pro δ -H₂, Gly α -H_AH_B*), 3.98-4.10 (1.82H, Gly α -H₂, Gly α -H_AH_B*), 4.47 (1H, t, J 7.5, Glu α -H) and 4.51-4.56 (1H, m, Pro α -H); δ_{C} (100 MHz; D_2O) 13.0 (CH₃, Glu γ -CH₂CH₂CH₂CH₃), 21.7 (CH₂, Glu γ -CH₂CH₂CH₂CH₃), 21.8* (CH₂, Glu γ -CH₂CH₂CH₂CH₃), 24.0 (CH₂, Pro γ -C), 28.1 (CH₂, Glu γ -CH₂CH₂CH₂CH₃), 28.2* (CH₂, Glu γ -CH₂CH₂CH₂CH₃), 29.3 (CH₂, Pro β -C), 30.9 (CH₂, Glu γ -CH₂CH₂CH₂CH₃), 31.3*, 31.6*, 32.1* (CH₂), 32.3 (CH₂, Glu β -C), 40.1* (CH₂, Gly α -C), 40.3 (CH₂, Gly α -C), 42.0 (CH, Glu γ -C), 43.0* (CH, Glu γ -C), 46.8 (CH₂, Pro δ -C), 47.4* (CH₂, Pro δ -C), 51.2 (CH, Glu α -C), 51.8* (CH, Glu α -C), 59.9* (CH, Pro α -C), 60.2 (CH, Pro α -C), 115.5 (q, J 291, CF₃), 162.9 (q, J 35.2, CF₃CO₂H), 165.5 (quat., Gly-CO), 166.0* (quat., Gly-CO), 173.5* (quat., CO), 174.0 (quat., CO), 174.5* (quat., CO), 174.8 (quat., CO), 180.0 (quat., Glu γ -CO) and 180.2* (quat., Glu γ -CO); m/z (FAB+) 358.1978 [MH(free base)⁺. C₁₆H₂₈N₃O₆ requires 358.1978].

Example 6: Synthesis of (2*S*, 9'*R*, 12'*S*)-2-[[[(1',4'-Diaza-2'-oxobicyclo[7.3.0]-dodecyl)-12'-carbonyl]amino]-1,5-pentanedioic acid trifluoroacetate salt

5



Scheme 1 Reagents, conditions and yields: (i) Grubbs catalyst, CH_2Cl_2 , reflux, 48 h, then DMSO, RT, 24 h (46%); (ii) PtO_2 , THF, H_2 , RT, 16 h then 30% HBr/HOAc, RT, 2 h then NaHCO_3 , H_2O , dioxane, Boc₂O, RT, 4 d (78%, 3 steps); (iii) BoP-Cl, Et_3N , CH_2Cl_2 , 0 °C to RT, 24 h (64%); (iv) $\text{CF}_3\text{CO}_2\text{H}$, CH_2Cl_2 , RT, 4 h (80%).

10

(6*Z*, 9*R*, 12*S*)-1,4-Diaza-4-benzyloxycarbonyl-12-*tert*-butoxycarbonyl-2-oxobicyclo[7.3.0]dodec-6-ene 65

To a degassed solution of diene 17 (0.11 g, 0.242 mmol) in dry dichloromethane (60 cm^3) was added bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs's catalyst) (0.020 g, 0.024 mmol) under an atmosphere of nitrogen and the resultant purple solution heated at reflux for 24 h. Further

bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs's catalyst) (0.020 g, 0.024 mmol) was then added, and refluxing continued for a further 24 h. The solution was cooled to room temperature, dimethyl sulphoxide (0.16 cm³, 2.26 mmol) was added and the orange/brown solution stirred for 22 h. The solvent was removed *in vacuo* and the residue purified by chromatography (SiO₂, hexanes-ethyl acetate, 3:1, 2:1, 1:1) to give an almost colourless oil which was further purified by chromatography (C₁₈ RP silica, 100:0, 9:1, 9:3, 1:1, 1:9, water-acetonitrile,) to give *alkene 65* (0.046 g, 46%) as a colourless oil. Alkene **65** existed exclusively as the *trans* C(O)-NPro conformer. In addition, restricted rotation about the N-CO carbamate bond was also observed resulting in a 1:1 mixture of conformers: [α]_D -243.9 (*c* 0.27 in CH₂Cl₂); δ_H (400 MHz; CDCl₃; Me₄Si) 1.49 [9H, s, C(CH₃)₃], 1.84-2.34 (5H, m, 8-*H_AH_B*, 10-H₂ and 11-H₂), 2.98-3.08 (1H, m, 8-*H_AH_B*), 3.69-3.88 (2H, m, 5-*H_AH_B* and 3-*H_AH_B*), 4.0 (1H, p, *J* 7.6, 9-H), 4.33-4.61 (3H, m, 5-*H_AH_B*, 3-*H_AH_B* and 12-H), 5.10-5.30 (2H, m, OCH₂Ph), 5.51-5.65 (1H, m, 6-H), 5.98-6.09 (1H, m, 7-H) and 7.29-7.38 (5H, m, Ph); δ_C (100 MHz; CDCl₃) 27.2 (CH₂, 11-C), 28.0 [CH₃, C(CH₃)₃], 33.9 (CH₂, 8-C), 35.1 (CH₂, 10-C), 42.2 (CH₂, 5-C), 42.3 (CH₂, 5-C), 45.7 (CH₂, 3-C), 45.9 (CH₂, 3-C), 59.5 (CH, 9-C), 59.6 (CH, 9-C), 61.2 (CH, 12-C), 61.3 (CH, 12-C), 67.4 (CH₂, OCH₂Ph), 67.5 (CH₂, OCH₂Ph), 81.4 [quat., C(CH₃)₃], 81.5 [quat., C(CH₃)₃], 125.8 (CH, 6-C), 126.0 (CH, 6-C), 127.7 (CH, Ph), 127.8 (CH, Ph), 127.81 (CH, Ph), 127.9 (CH, Ph), 128.3 (CH, Ph), 132.0 (CH, 7-C), 132.4 (CH, 7-C), 136.5 (quat., Ph), 155.8 (quat., NCO₂), 156.2 (quat., NCO₂), 167.2 (quat., 2-C), 167.4 (quat., 2-C), 171.2 (quat., 12-CO) and 171.4 (quat., 12-CO); *m/z* (EI+) 414.2154 (*M*⁺. C₂₃H₃₀N₂O₅ requires 414.2154).

(9R, 12S)-1,4-Diaza-4-tert-butylloxycarbonyl-2-oxobicyclo[7.3.0]dodec-2-carboxylic acid 66

To a stirred solution of alkene **65** (0.12 g, 0.29 mmol) in tetrahydrofuran (4 cm³) was added platinum oxide (0.0066 g, 0.029 mmol) under a flow of

nitrogen. The mixture was hydrogenated (1 atm of H₂) for 16 h, filtered through Celite™, and the solvent removed *in vacuo*. The residue was dissolved in a solution of hydrobromic acid in acetic acid (30% w/w, 3 cm³) and stirred at room temperature for 2 h. Removal of the volatiles *in vacuo* at 30 °C followed by addition and evaporation of methanol:water (3:1) several times yielded the hydrobromide salt which was dissolved in a solution of saturated sodium hydrogen carbonate (3 cm³). Dioxane (2 cm³) was then added and di-*tert*-butyl dicarbonate added to the opaque solution (0.076 g, 0.350 mmol). The resultant suspension was stirred for 24 h, di-*tert*-butyl dicarbonate (0.076 g, 0.3504 mmol), added and stirring continued for a further 72 h. Water (10 cm³) was added, the solution washed with dichloromethane, and the aqueous layer acidified with 2 M aqueous hydrochloric acid and extracted with dichloromethane. The combined organic layers were dried (Na₂SO₄), filtered and the solvent removed to yield an oil (0.08 g) that was purified by chromatography (SiO₂, 2:1:0, 2:1:0.3, hexanes-ethyl acetate-acetic acid,) to give acid 66 (0.074 g, *ca.* 78%) as a colourless oil. Acid 66 existed exclusively as the *trans* C(O)-NPro conformer. In addition, restricted rotation about the N-CO carbamate bond was also observed resulting in a 1:1 mixture of conformers: δ_H (400 MHz; CDCl₃; Me₄Si) 1.25-1.48 [11H, m, C(CH₃)₃, 8-*H_AH_B* and 6-*H_AH_B* or 7-*H_AH_B*], 1.54-1.82 (5H, m, 8-*H_AH_B*, 10-*H_AH_B* and 6-*H_AH_B* or 7-*H_AH_B*), 1.90-2.03 (1H, m, 10-*H_AH_B*), 2.10-2.33 (2H, m, 11-H₂), 2.78 (1H, td, *J* 12.7 and 2.6, 5-*H_AH_B*), 3.78-3.95 (2H, m, 5-*H_AH_B* and 3-H), 4.20-4.30 (1H, m, 9-H), 4.52 (0.5H, d, *J* 17.7, 3-*H_AH_B*), 4.62 (1H, t, *J* 8.6, 12-H) and 4.76 (0.5H, d, *J* 17.2, 3-*H_AH_B*); δ_C (100 MHz; CDCl₃) 22.2 (CH₂, 6-C or 7-C), 27.6 (CH₂, 6-C or 7-C), 24.3 (CH₂, 11-C), 24.8 (CH₂, 11-C), 26.8 (CH₂, 6-C or 7-C), 27.9 (CH₂, 6-C or 7-C), 28.3 [CH₃, C(CH₃)₃], 32.6 (CH₂, 10-C), 33.1 (CH₂, 10-C), 34.8 (CH₂, 8-C), 35.3 (CH₂, 8-C), 51.1 (CH₂, 5-C), 51.7 (CH₂, 5-C), 54.7 (CH₂, 3-C), 55.9 (CH₂, 3-C), 56.6 (CH, 9-C), 56.7 (CH, 9-C), 60.8 (CH, 12-C), 61.1 (CH, 12-C), 80.7 [quat., C(CH₃)₃], 154.8 (quat., NCO₂), 155.6 (quat., NCO₂), 170.0 (quat.,

2-C), 170.5 (quat., 2-C), 174.1 (quat., 12-CO) and 174.7 (quat., 12-CO); m/z (FAB+) 327.1925 (MH^+ . $C_{16}H_{27}N_2O_5$ requires 327.1920).

5 *(2S, 9'S, 12'S)-Di-tert-butyl 2-[[[(1',4'-diaz-4'-tert-butyloxy)carbonyl]-2'-oxobicyclo[7.3.0]dodecyl]-12'-carbonyl]amino}-1,5-pentanoate 67*

Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoP-Cl) (0.08 g, 0.31 mmol) was added to a solution of acid 66 (0.07 g, 0.20 mmol), L-glutamic acid di-tert-butyl ester hydrochloride 21 (0.085 g, 0.289 mmol) and triethylamine (0.084 cm³, 0.60 mmol) in dichloromethane (7 cm³) at 0 °C. The mixture was
 10 stirred for 24 h, washed with saturated aqueous sodium hydrogen carbonate, 2 M aqueous hydrochloric acid, dried (Na₂SO₄), filtered and the solvent removed under reduced pressure. The resultant oil (0.141 g) was purified by chromatography (SiO₂, 1:1, 1:2, hexane-ethyl acetate) to afford *amide 67* (0.077 g, ca. 64%) as a colourless oil. Amide 67 existed exclusively as the *trans* C(O)-
 15 NPro conformer. In addition, restricted rotation about the N-CO carbamate bond was also observed resulting in a 46:54 mixture of conformers: δ_H (300 MHz; CDCl₃; Me₄Si) 1.14-1.30 [2H, m, 8'-H_AH_B and H'-6(1H) or H'-7(1H)], 1.34-1.41 [27H, m, 3 x C(CH₃)₃], 1.54-2.28 (11H, m, 8'-H_AH_B, 10'-H₂, 3-H₂, 4-H₂, 6'-H(3H) or 7'-H(3H)] and 11'-H_AH_B), 2.31-2.49 (1H, m, 11'-H_AH_B), 2.60-
 20 2.70 (1H, m, 5'-H_AH_B), 3.65-3.87 (2H, m, 5'-H_AH_B and 3'-H_AH_B), 4.18 (1H, br s, 9'-H), 4.39 (1H, q, J 7.6, 2-H), 4.48 (0.5H, d, J 17.5, 3'-H_AH_B), 4.65 (1H, t, J 7.8, 12'-H), 4.74 (0.5H, d, J 17.0, 3'-H_AH_B), 7.45 (0.46H, d, J 7.4, N-H), and 7.64 (0.54H, d, J 7.7, N-H); δ_C (75 MHz; CDCl₃) 22.7 (CH₂, 11'-C), 23.3 (CH₂, 6'-C or 7'-C), 23.4 (CH₂, 6'-C or 7'-C), 27.0 (CH₂, 3-C, 6'-C or 7'-C),
 25 27.7 (CH₂, 3-C, 6'-C or 7'-C), 27.8 (CH₂, 3-C, 6'-C or 7'-C), 28.2 (CH₂, 3-C, 6'-C or 7'-C), 27.9 [CH₃, C(CH₃)₃], 28.0 [CH₃, C(CH₃)₃], 28.4 [CH₃, C(CH₃)₃], 31.4 (CH₂, 4-C), 32.5 (CH₂, 10'-C), 32.8 (CH₂, 10'-C), 34.4 (CH₂, 8'-C), 35.1 (CH₂, 8'-C), 50.7 (CH₂, 5'-C), 51.5 (CH₂, 5'-C), 52.25 (CH, 2-C), 52.33 (CH, 2-C), 54.6 (CH₂, 3'-C), 55.0 (CH₂, 3'-C), 56.6 (CH, 9'-C), 56.8 (CH, 9'-C),
 30 60.2 (CH, 12'-C), 60.8 (CH, 12'-C), 80.4 [quat., C(CH₃)₃], 80.6 [quat.,

C(CH₃)₃], 81.9 [quat., C(CH₃)₃], 154.7 (quat., NCO₂), 155.5 (quat., NCO₂), 169.4 (quat., 2'-C), 169.8 (quat., 2'-C), 170.6 (quat., 12'-CO, 1-C, 5-C), 170.8 (quat., 12'-CO, 1-C, 5-C) and 171.8 (quat., 12'-CO, 1-C, 5-C); *m/z* (FAB+) 568.3592 (MH⁺. C₂₉H₅₀N₃O₈ requires 568.3598).

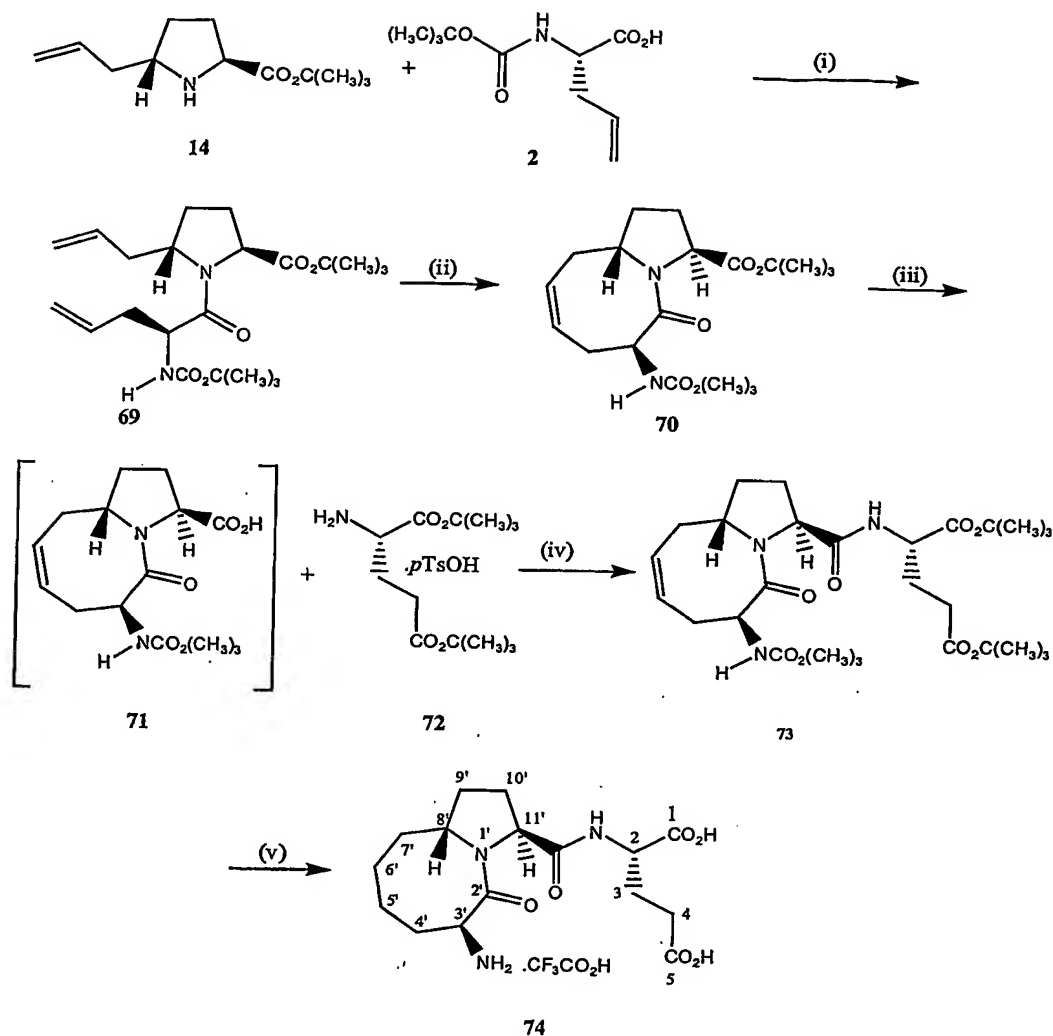
5

(2*S*, 9'*R*, 12'*S*)-2-[[*(1',4'*-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino]-1,5-pentanedioic acid trifluoroacetate **68**

Amide **67** (0.072 g, 0.126 mmol) was dissolved in a mixture of dichloromethane and trifluoroacetic acid (5:2, v/v) and stirred at room temperature for 4 h. Evaporation of the volatiles, and subsequent purification by RP-HPLC [water (0.05% trifluoroacetic acid):acetonitrile, 90:10, 13 ml min⁻¹], gave **68** (0.059 g, 80%) as a white solid after drying on a freeze drier. Macrocycle **68** existed exclusively as the *trans* C(O)-NPro conformer: mp[†] 30-130 °C; [α]_D -36.9 (c 0.195 in MeOH); δ_H (400 MHz; CDCl₃; Me₄Si) 1.71-2.29 (11H, m, 6'-H₂, 7'-H₂, 8'-H₂, 10'-H₂, 3-H₂ and 11'-H_AH_B), 2.33-2.39 (1H, m, 11'-H_AH_B), 3.27 (1H, dt, *J* 10.2 and 4.1, 5'-H_AH_B), 3.39 (1H, td, *J* 12.0 and 2.2, 5'-H_AH_B), 3.76 (1H, d, *J* 13.6, 3'-H_AH_B), 4.25-4.20 (2H, m, 3'-H_AH_B and 9'-H), 4.45 (1H, dd, *J* 9.3 and 5.1, 2-H) and 4.51 (1H, dd, *J* 9.2 and 8.3, 12'-H); δ_C (100 MHz; CDCl₃) 24.06 (CH₂, 6'-C or 7'-C), 24.1 (CH₂, 6'-C or 7'-C), 25.7 (CH₂, 3-C), 27.7 (CH₂, 11'-C), 29.6 (CH₂, 4-C), 32.5 (CH₂, 10'-C), 33.4 (CH₂, 8'-C), 43.3 (CH₂, 5'-C), 46.8 (CH₂, 3'-C), 51.8 (CH, 2-C), 61.9 (CH, 12'-C), 62.8 (CH, 9'-C), 116.2 (quat., q, *J* 290, CF₃), 162.6 (quat., q, *J* 35.2, CF₃CO₂H), 164.7 (quat., 2'-C), 173.4 (quat., 12'-CO), 174.6 (quat., 1-C), and 176.8 (quat., 5-C); *m/z* (FAB+) 356.1823 [MH(free base)⁺. C₁₆H₂₆N₃O₆ requires 356.1822].

25

Example 7: *Synthesis of (2S, 3'S, 8'S, 11'S)-2-[[[(3'-Amino-1'-aza-2'-oxo-bicyclo[6.3.0]undecyl)-11'-carbonyl]amino]-1,5-pentanedioic acid trifluoroacetate salt*



Scheme 1 Reagents, conditions and yields: (i) DCC, CH_2Cl_2 , 0 °C to RT, 18 h (81%); (iii) Grubbs' catalyst, CH_2Cl_2 , 45 °C, 4 d then dmsO, RT, overnight (39%); (iii) $\text{CF}_3\text{CO}_2\text{H}$, CH_2Cl_2 , RT, 5 h then NaHCO_3 , H_2O , dioxane, Boc_2O , RT, 21 h; (iv) BoP-Cl , Et_3N , CH_2Cl_2 , 0 °C to RT, 19 h (60%, over 3 steps); (v) PtO_2 , EtOAc , H_2 , $\text{CF}_3\text{CO}_2\text{H}$, RT, 6 h then PtO_2 , THF/MeOH , H_2 , RT, overnight, then CH_2Cl_2 , $\text{CF}_3\text{CO}_2\text{H}$, RT, 4 h (12%).

(2*S*, 5*S*)-*N*-tert-Butoxycarbonyl- α -allylglycine-5-allyl-*L*-proline tert-butyl ester

N,N'-Dicyclohexylcarbodiimide (0.057 g, 0.2761 mmol) was added to a stirred solution of amine 14 (0.053 g, 0.251 mmol) and *N*-*tert*-butoxycarbonyl- α -allylglycine 2 (0.059 g, 0.2761 mmol) in dichloromethane (5 cm³) and the mixture stirred overnight, then filtered through Celite™ to remove DCU. The reaction mixture was subsequently washed with saturated aqueous sodium hydrogen carbonate, 2M aqueous hydrochloric acid, dried (MgSO₄), filtered and the solvent removed to yield an oil (0.106 g) which was purified by chromatography (silica gel, hexane:ethyl acetate, 7:1, 5:1) to afford *diene* 69 (0.083 g, 81%) as a colourless oil. Due to the complexity of the NMR spectra, the data is not reported; *m/z* (FAB+) 409.2707 (MH⁺. C₂₂H₃₇N₂O₅ requires 409.2702).

(8S, 11S)-1-Aza-2-oxo-3-tert-butoxycarbonylamino-11-tert-butoxycarbonylbicyclo[6.3.0]undec-5-ene 70

To a degassed solution of *diene* 69 (0.053 g, 0.129 mmol) in dry dichloromethane (32 cm³) was added bis(tricyclohexylphosphine)benzylideneruthenium dichloride (Grubbs' catalyst) (0.011 g, 0.0129 mmol) under a flow of nitrogen and the resultant purple solution heated at reflux under a nitrogen atmosphere for 24 h. Further bis(tricyclohexylphosphine)benzylideneruthenium dichloride (Grubbs' catalyst) (0.011 g, 0.0129 mmol) was added and refluxing continued for a further 48 h after which time another portion of Grubbs catalyst (0.011 g, 0.0129 mmol) was added and the solution refluxed for an additional 24 h. The orange/brown solution was cooled to room temperature, dimethyl sulphoxide (0.151 cm³, 1.935 mmol) was added and the solution stirred overnight. The solvent was removed *in vacuo* and the residue purified by chromatography (silica gel, hexanes:ethyl acetate, 4:3, 3:1, 2:1) to give *alkene* 70 (0.0237 g, 39%) as an off-white solid. *Alkene* 70 was shown to be exclusively *trans* C(O)-NPro conformer: mp 175-195 °C; [α]_D -24.9 (*c* 0.237 in MeOH); δ_H (400 MHz; CDCl₃; Me₄Si) 1.45 [9H, s, C(CH₃)₃], 1.47 [9H, s, C(CH₃)₃], 1.68-1.74 (1H, m,

9- H_AH_B), 1.82 (1H, td, J 11.0 and 5.9, 10- H_AH_B), 2.17-2.26 (2H, m, 9- H_AH_B and 10- H_AH_B), 2.27-2.40 (1H, br m, 7- H_AH_B), 2.49 (1H, br d, J 17.6, 7- H_AH_B), 2.55-2.63 (1H, m, 4- H_AH_B), 2.66-2.80 (1H, m, 4- H_AH_B), 4.40 (1H, dd, J 8.6 and 4.2, 11-H), 4.45-4.62 (2H, m, 3-H and 8-H), 5.62-5.68 (2H, m, H-6 and N-H) and 5.80-5.90 (1H, m, 5-H); δ_C (100 MHz; $CDCl_3$) 25.5 (CH_2 , 10-C), 27.9 [CH_3 , $C(CH_3)_3$], 28.3 [CH_3 , $C(CH_3)_3$], 32.6 (CH_2 , 4-C), 33.4 (CH_2 , 7-C and 9-C), 55.8 (CH, 3-C), 57.2 (CH, 8-C), 61.5 (CH, 11-C), 79.5 [quat., $C(CH_3)_3$], 81.2 [quat., $C(CH_3)_3$], 128.6 (CH, 5-C and 6-C), 155.1 (quat., NCO_2), 169.6 (quat., 2-C) and 171.2 (quat., 11-CO); m/z (EI+) 380.2304 (M^+ . $C_{20}H_{32}N_2O_5$ requires 380.2311).

10

(2*S*, 8'*S*, 11'*S*)-Di-*tert*-butyl 2-[[*(1-Aza-2-oxo-3-tert-butoxycarbonylaminobicyclo[6.3.0]undec-5-ene)-11-carbonyl*]]amino}-1,5-pentanedioate **73**

Alkene **70** (0.021 g, 0.055 mmol) was stirred at room temperature in dichloromethane:trifluoroacetic acid (3:1, v/v, 4 cm^3) for 5 h. Removal of the volatiles *in vacuo* yielded an oil that was dissolved in saturated aqueous sodium hydrogen carbonate (1.5 cm^3). Dioxane (1 cm^3) was added followed by di-*tert*-butyl dicarbonate (0.015 g, 0.066 mmol) and the milky suspension was stirred at room temperature for 21 h. The reaction was diluted with water until a solution was obtained and extracted with dichloromethane. The aqueous layer was acidified with 2M aqueous hydrochloric acid, extracted with dichloromethane and the combined organic extracts were dried (Na_2SO_4). Removal of the solvent *in vacuo* gave acid **71** (0.012 g) that was dissolved in dichloromethane (3 cm^3). Glutamic acid di-*tert*-butyl ester hydrochloride **72** (0.013 g, 0.044 mmol) was added and the solution cooled to 0 °C. Triethylamine (0.013 cm^3 , 0.092 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoP-Cl) (0.012 g, 0.048 mmol) were added and the mixture stirred for 19 h. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate, 2M aqueous hydrochloric acid, dried (Na_2SO_4) and the solvent removed to yield an oil (0.033 g) which was purified by chromatography (silica gel, hexane:ethyl

acetate, 2:1, 1:1, 1:2) to afford *amide 73* (0.0125 g, 60%, 3 steps) as a colourless oil: $[\alpha]_D^{25} +12.1$ (c 0.247 in CH_2Cl_2); δ_H (400 MHz; CDCl_3 ; Me_4Si) 1.45-1.48 [27H, s, 3 x $\text{C}(\text{CH}_3)_3$], 1.65-1.75 (1H, m, 9'- H_AH_B), 1.87-1.96 (1H, m, 3'- H_AH_B), 2.02-2.41 (7H, m, 10'- H_2 , 4'- H_2 , 3'- H_AH_B , 9'- H_AH_B and 7'- H_AH_B), 2.51 (1H, br d, J 17.4, 7'- H_AH_B), 2.57-2.64 (1H, m, 4'- H_AH_B), 2.70-2.80 (1H, m, 4'- H_AH_B), 4.39-4.48 (2H, m, 11'-H and 2-H), 4.52-4.62 (2H, m, 8'-H and 3'-H), 5.63-5.69 (1H, m, 6'-H), 5.78-5.84 (1H, m, 5'-H), 5.91 (1H, br s, N-H) and 6.73 (1H, d, J 7.0, N-H); δ_C (100 MHz; CDCl_3) 25.4 (CH_2 , 10'-C), 27.2 (CH_2 , 3-C), 27.9 [CH_3 , $\text{C}(\text{CH}_3)_3$], 28.0 [CH_3 , $\text{C}(\text{CH}_3)_3$], 28.3 [CH_3 , $\text{C}(\text{CH}_3)_3$], 31.3 (CH_2 , 4-C), 32.5 (CH_2 , 5'-C), 33.3 (CH_2 , 9'-C), 33.7 (CH_2 , 7'-C), 52.3 (CH, 2-C), 56.3 (CH, 3'-C), 57.8 (CH, 8'-C), 61.9 (CH, 11'-C), 79.7 [quat., $\text{C}(\text{CH}_3)_3$], 80.6 [quat., $\text{C}(\text{CH}_3)_3$], 82.0 [quat., $\text{C}(\text{CH}_3)_3$], 127.9 (CH, 5'-C), 128.6 (CH, 6'-C), 155.4 (quat., NCO_2), 170.4 (quat., 2'-C), 170.8 (quat., CO), 171.7 (quat., CO) and 172.6 (quat., CO);

15

(2*S*, 3'*S*, 8'*S*, 11'*S*)-2-[(3'-Amino-1'-aza-2'-oxo-bicyclo[6.3.0]undecyl)-11'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt **74**

PtO_2 (0.001 g, 0.0042 mmol) was added to a stirred solution of *amide 73* (0.012 g, 0.021 mmol) in ethyl acetate:trifluoroacetic acid (5:3, v/v, 8 cm^3) under a nitrogen atmosphere. The mixture was hydrogenated (1 atm. of hydrogen) overnight, filtered through Celite™, and the solvent removed *in vacuo*. NMR and HPLC analysis showed the reaction to be incomplete (both double bond and protecting groups remaining). Thus PtO_2 (0.0003 g, 0.0126 mmol) was added to a stirred solution of the residue in tetrahydrofuran:methanol (1:1, v/v, 2 cm^3) under a nitrogen atmosphere. The mixture was hydrogenated (1 atm. of hydrogen) overnight, filtered through Celite™, and the solvent removed *in vacuo*. The residue was dissolved in dichloromethane (3 cm^3), trifluoroacetic acid (1 cm^3) added and the solution stirred for 4 h at room temperature. Removal of the volatiles *in vacuo*, purification by RP HPLC [90% water (containing 0.05% trifluoroacetic

acid):10% acetonitrile, 13 ml min⁻¹] and drying on a freeze drier gave **74** (1.4 mg 12%, from **73**) as a thin film. Due to the small amount of sample obtained an optical rotation was not recorded: δ_H (400 MHz; D₂O) 1.74-2.42 (14H, m, 5'-H₂, 6'-H₂, 4'-H₂, 9'-H₂, 3-H₂, 7'-H₂ and 10'-H₂), 2.52 (2H, t, *J* 8.8, 4-H₂), 4.3-4.4 (1H, m, 2H), 4.43-4.5 (1H, m) and 4.62-4.69 (1H, m); δ_C (100 MHz; D₂O) 22.3 (CH₂), 25.4 (CH₂), 28.4 (CH₂), 29.0 (CH₂), 30.2 (CH₂), 32.4 (CH₂), 33.7 (CH₂), 53.9 (CH), 55.1 (CH), 60.2 (CH), 62.8 (CH), 168.1 (quat., CO) and 173.7 (quat., CO); Note: due to small amount of sample 2 quaternary carbons and trifluoroacetate signals not detected; *m/z* (FAB+) 356.1816 [MH(free base)]⁺. C₁₆H₂₆N₃O₆ requires 356.1822].

***In Vitro* Testing**

The following Examples are provided to demonstrate features of this invention. They are not intended to be limiting, and other compositions and methods of this invention can be developed without undue experimentation. All of those compositions and methods are considered to be part of this invention. All references cited herein are incorporated fully by reference.

All the following experiments were carried out using protocols developed under guidelines approved by the University of Auckland Animal Ethics Committee.

Example 8: Effects of Cyclic G-2AllylP on Cerebellar Cell Explants

To determine the effects of the compounds of the invention on neuronal cells *in vitro*, a series of studies was carried out using cerebellar explants from adult rats. *In vitro* systems are suitable for studying neuronal proliferation, neurite growth, formation of nerve bundles and effects of toxins on neural cells, effects that parallel effects observed *in vivo*. Thus, results of studies using *in vitro* cerebellar explants are predictive of effects of interventions *in vivo*.

In a first series of studies, effects of glutamate on cerebellar explants were determined. At physiological concentrations, glutamate is a

neurotransmitter in the CNS of mammals, including humans. However, at sufficiently high concentrations, glutamate is neurotoxic, resulting in neuronal cell death. Because glutamate is a naturally occurring neurotransmitter in the CNS of mammals, including humans, and because glutamate neurotoxicity is recognized in the art as reflective of neurotoxicity in general, and including cell death and degeneration, it is a valuable tool useful for identifying and characterizing agents effective in treatment of neurodegeneration and neural cell death.

Materials and Methods

Cover slips were placed into a large Petri dish and washed in 70% alcohol for 5 minutes, then washed with Millipore H₂O. The cover slips were air dried, and coated with Poly-D-Lysine (1 mg/ml stock solution in PBS, 90-100 μ l) for 2 hours at 34°C.

Extraction of Cerebellar Tissue

Postnatal day 8 Wistar rats were used for the study. The rats were sacrificed and placed in ice for 1 minute, decapitated and the cerebellum removed and placed on ice. Cerebellum tissue was placed in 1 ml of 0.65% glucose-supplemented PBS (10 μ l 65% stock D (+)glucose/1ml PBS) in a large Petri dish, chopped up into smaller sections and triturated with a 1ml insulin syringe via a 23 G (0.4mm) needle, and then squirted back into the glucose solution in the large Petri dish. The tissue was sieved through (125 μ m pore size gauze) and centrifuged (2 minutes at 60g) twice to exchange the medium into serum-free BSA-supplemented START V medium (Biochrom, Germany). The second centrifugation step was done with 1ml of START V medium. The microexplants were reconstituted into 500 μ l of START V medium and put on ice.

Cultivation of Cerebellar Cells

Two hours after PDL-coating, the slides were washed with Millipore H₂O and air dried. Each slide was placed into a small Petri dish (diameter: 35 mm) and 40 μ l of START V/cell suspension was added. The tissue was
5 incubated for 2 hours at 34°C (settlement period). START V-medium (1ml) was then added to the Petri dish and cultivated at 34° C in the presence of 5% CO₂ in air at 100% humidity for 48 hours.

Drug Application

For the study, certain explant cultures were exposed to vehicle (PBS)
10 only. In the first study (Study 1) 10 μ l of toxin 1 (L-glutamate- 100mM in Millipore water; final concentration: 1mM) and 10 μ l of toxin 2 (3-nitropropionic acid- 50mM- pH 7- in Millipore water, final concentration: 0.5mM) was applied simultaneously with the compounds to be tested (10mM stock solution prepared in PBS and diluted to final concentrations between 1-
15 100 nM). In each case, the drugs were left in contact with the explants for the duration of the study.

Methods for Determining Drug Effects

After explants were exposed to drugs for the study period, cells were
20 then rinsed in PBS and then fixed in increasing concentrations of paraformaldehyde (500 μ l of 0.4% PFA was applied; then 1.2% PFA; then 3% PFA and finally 4% PFA (each fixation step: 2-3 minutes). Finally, the microexplants were rinsed in PBS.

Neurons in the explants were then evaluated for morphology (presence
25 of neurites) and counted as live cells per microscopic field. Four fields displaying highest cell density were counted per cover slip and the data presented as mean \pm standard error of the mean (SEM); n= 4 each. Statistical significance was evaluated by using the non-paired Student's t-test.

Results

The results of the study are shown in Figure 1 and Figure 2. Glutamate treatment (1 mM) resulted in about an 80% loss of cerebellar neurons having neurites compared to vehicle-treated controls. In contrast, administration of (2*S*, 3'*S*, 8'*R*, 11'*S*) 2-{[(3'-Amino-1'-aza—2'-oxobicyclo[6.3.0]-undecyl)-11'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 48 and (2*S*, 9'*R*, 12'*S*)-2-{[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate 68 (Figures 1 and 2 respectively) significantly increased the numbers of cells having neurites in a dose-dependent manner when administered simultaneously with glutamate. Treatment with (2*S*, 3'*S*, 8'*R*, 11'*S*) 2-{[(3'-Amino-1'-aza—2'-oxobicyclo[6.3.0]-undecyl)-11'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 48 and (2*S*, 9'*R*, 12'*S*)-2-{[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate 68 (Figures 1 and 2 respectively) showed a significant recovery from glutamate-induced neurotoxicity.

Conclusions

Treatment with (2*S*, 3'*S*, 8'*R*, 11'*S*) 2-{[(3'-Amino-1'-aza—2'-oxobicyclo[6.3.0]-undecyl)-11'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 48 and (2*S*, 9'*R*, 12'*S*)-2-{[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate 68 prevented glutamate-induced neurotoxicity, indicating that (2*S*, 3'*S*, 8'*R*, 11'*S*) 2-{[(3'-Amino-1'-aza—2'-oxobicyclo[6.3.0]-undecyl)-11'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 48 and (2*S*, 9'*R*, 12'*S*)-2-{[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate 68 are neuroprotective and can be used to reverse and/or inhibit neuronal degeneration or cell death.

Example 9: Hypoxic-Ischemic Injury

Materials and Methods

To determine whether compounds of the invention might prevent neuronal injury in response to stroke, cardiac arterial bypass graft surgery (CABG) or other hypoxic insults, a series of studies were carried out in rats that had been exposed to hypoxic-ischemic injury (HI). Adult rats (Wistar, 280-310g, male) were used. The modified Levine model preparation and experimental procedures were used (Rice *et al*, 1981, *Ann. Neurol.* :9: 131-141; Guan *et al J.*, 1993, *Cereb. Blood Flow Metab.*: 13(4): 609-16). These procedures in brief, consist of an HI injury induced by unilateral carotid artery ligation followed by inhalational asphyxia in the animals with an implanted lateral ventricular cannula. A guide cannula was stereotaxically placed on the top of the dura 1.5mm to the right of the mid-line and 7.5mm anterior to the interaural zero plane under halothane anaesthesia. The right carotid artery was double ligated two days after the cannulation. After 1 hour recovery from the anaesthesia, each of the rats were placed in an incubator where the humidity (90±5%) and temperature (31°±0.5°C) were controlled for another hour, then exposed to hypoxia (6% oxygen) for 10 min. The animals were kept in the incubator for an additional 2 hours before treatment.

Pairs of rats were treated intracerebral ventricularly (icv) with either (2S, 9'R,12'S)-2-[[[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino]-1,5-pentanedioic acid trifluoroacetate (68) (100 pM) or vehicle (normal saline) 2 hours after hypoxic-ischemic insult. Rats in each group were simultaneously infused with (2S, 9'R, 12'S)-2-[[[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino]-1,5-pentanedioic acid trifluoroacetate (68) or vehicle under light anaesthesia (1.5% halothane) 2 hours after the insult. A total volume of 20µl was infused (icv) over 20 minutes by a micro-infusion pump.

Histological examination was performed on rats 5 days after the hypoxic-ischemic injury. The rats were killed with an overdose of sodium pentobarbital and were perfused transcardially with normal saline followed by

10% formalin. The brains were kept in the same fixative for a minimum of 2 days before being processed using a standard paraffin imbedding procedure.

Coronal sections 8 μ m in thickness were cut from the striatum, cerebral cortex and hippocampus and were stained with thionin and acid fuchsin. The histological outcome was assessed at three levels: (1) the mid level of the striatum, (2) where the completed hippocampus first appeared and (3) the level where the ventral horn of the hippocampus just appears. The severity of tissue damage was scored in the striatum, cortex and the CA1-2, CA3, CA4 and dentate gyrus of the hippocampus. Tissue damage was identified as neuronal loss (acidophilic (red) cytoplasm and contracted nuclei), pan-necrosis and cellular reactions. Tissue damage was scored using the following scoring system: 0: tissue showed no tissue damage, 1: <5% tissue was damaged, 2: <50% tissue was damaged, 3: >50% tissue was damaged and 4: >95% tissue was damaged.

Results and Conclusion

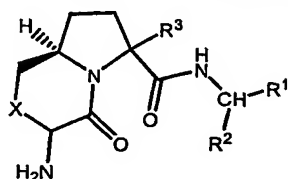
The results of this study are shown in FIG.3. FIG.3 shows that hypoxic-ischemic injury (left bars of each set) resulted in significant damage scores in each of the areas of the brain studied. FIG.3 also shows that central administration of a relatively low dose of (2S, 9'R, 12'S)-2-[[[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate (68) (right bars of each set; 100 pM) significantly reduced the tissue damage in each brain region examined compared to the vehicle treated group ($p < 0.001$).

It can be seen that (2S, 9'R, 12'S)-2-[[[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate (68) can be neuroprotective against neural damage caused by hypoxic-ischemic injury, even when administered after hypoxic-ischemic injury. This surprising finding indicates that (2S, 9'R, 12'S)-2-[[[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-pentanedioic acid

trifluoroacetate (68) is a useful agent to treat a variety of conditions characterized by neural degeneration or cell death.

What is claimed is:

1. A compound having the formula:



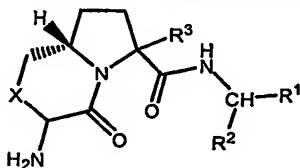
- 5 or a pharmaceutically acceptable salt or hydrate thereof, wherein

R^1 and R^2 are independently selected from the group consisting of $-OR'$, $-SR'$, $-NR'R'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R'$, $-C(NR')NR'R'$, trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of $-H$, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

R^3 is selected from the group consisting of $-H$, $-OR'$, $-SR'$, $-NR'R'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R'$, $-C(NR')NR'R'$, trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of $-H$, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C_0-C_3 .

2. A compound having the formula:



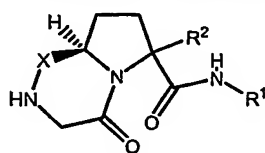
or a pharmaceutically acceptable salt or hydrate thereof, wherein

R^1 and R^2 are independently selected from the group consisting of $-OR'$, $-SR'$, $-NR'R'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R'$, $-C(NR')NR'R'$, trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of $-H$, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

R^3 is selected from the group consisting of $-H$, $-OR'$, $-SR'$, $-NR'R'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R'$, $-C(NR')NR'R'$, trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of $-H$, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C_0-C_3 .

3. A compound having the formula:



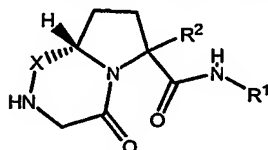
or a pharmaceutically acceptable salt or hydrate thereof, wherein

R^1 and R^2 are independently selected from the group consisting of $-H$, $-OR'$, $-SR'$, $-NR'R'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R'$, $-C(NR')NR'R'$, trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is

independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₄.

- 5 4. A compound having the formula:

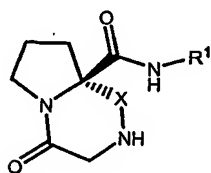


or a pharmaceutically acceptable salt or hydrate thereof, wherein

- R¹ and R² are independently selected from the group consisting of -H, -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₄.

5. A compound having the formula:



- 20 or a pharmaceutically acceptable salt or hydrate thereof, wherein

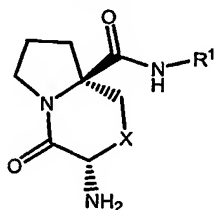
- R is independently selected from the group consisting of -H, -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted

arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₄.

5

6. A compound having the formula:

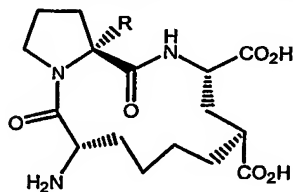


or a pharmaceutically acceptable salt or hydrate thereof, wherein

R is independently selected from the group consisting of -H, -OR', -SR',
 10 -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R',
 trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted
 heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl,
 substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted
 arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is
 15 independently selected from the group consisting of -H, alkyl, heteroalkyl,
 alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl and;

X is a linear alkyl or alkenyl chain of C₀-C₄.

7. A compound having the formula:



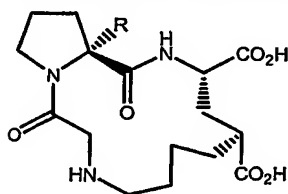
20

or a pharmaceutically acceptable salt or hydrate thereof, wherein

R is independently selected from the group consisting of -H, -OR', -SR',
 -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R',

- trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is
- 5 independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl.

8. A compound having the formula:



- 10 or a pharmaceutically acceptable salt or hydrate thereof, wherein

- R is independently selected from the group consisting of -H, -OR', -SR', -NR'R', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R', -C(NR')NR'R', trihalomethyl, halogen, alkyl, substituted alkyl, heteroalkyl, substituted heteroalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, heteroarylalkyl and substituted heteroarylalkyl; each R' is
- 15 independently selected from the group consisting of -H, alkyl, heteroalkyl, alkenyl, alkynyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl.

- 20 9. A compound of Formula 1 where R¹ = -COOH and R² = -(CH₂)₂-COOH.

10. A compound of Formula 3 where R¹ = -CH(COOH)-(CH₂)₂-COOH.

- 25 11. A compound of Formula 1 where R³ = methyl or Formula 3 where R² = methyl.

12. A compound of Formula 1 where $R^3 = \text{allyl}$ or Formula 3 where $R^2 = \text{allyl}$.
13. A compound of Formula 1 or Formula 3 where $X = -(\text{CH}_2)_3-$.
14. A compound of Formula 1 where $X = -(\text{CH}_2)_2-$.
15. A compound of Formula 3 where $X = -(\text{CH}_2)_4-$.
16. A compound of Formula 1 where $X = -\text{CH}_2-\text{CH}=\text{CH}-$, (where the CH_2 of X is adjacent to the carbon attached to the NH_2 group).
17. A compound of Formula 3 where $X = -\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$.
18. A compound of Formula 1 where $R^1 = -\text{COOH}$; $R^2 = -(\text{CH}_2)_2-\text{COOH}$; $R^3 = \text{H}$; $X = -(\text{CH}_2)_3-$.
19. A compound of Formula 3 where $R^1 = -\text{CH}(\text{COOH})-(\text{CH}_2)_2-\text{COOH}$; $R^2 = \text{H}$; $X = -(\text{CH}_2)_4-$.
20. A pharmaceutical composition comprising a compound of one or more of Formula 1, Formula 2, Formula 3 or Formula 4, Formula 5, Formula 6, Formula 7 or Formula 8 and a pharmaceutically acceptable excipient.
21. A pharmaceutical composition comprising a compound of one or more of Formula 1, Formula 2, Formula 3 or Formula 4, Formula 5, Formula 6, Formula 7 or Formula 8, a pharmaceutically acceptable excipient and a binder.

22. A pharmaceutical composition comprising a compound of one or more of Formula 1, Formula 2, Formula 3 or Formula 4, Formula 5, Formula 6, Formula 7 or Formula 8, a pharmaceutically acceptable excipient and a capsule.
- 5 23. A method of treating a patient to protect neurons otherwise destined to degenerate or die as a result of an injury or disease, comprising administering to a patient an effective amount of one or more compounds of Formula 1, Formula 2, Formula 3 or Formula 4, Formula 5, Formula 6, Formula 7 or Formula 8.
- 10 24. The method of claim 23, wherein the disease is characterized by apoptotic neuronal death.
25. The method of claim 23, wherein the disease is characterized by necrotic neuronal cell death.
- 15 26. The method of claim 23, wherein the disease is characterized by neuronal cell degeneration.
27. The method of claim 23, wherein the injury results in necrotic neuronal cell death.
- 20 28. The method of claim 23, wherein the injury results in apoptotic neuronal cell death.
- 25 29. The method of claim 23, wherein the injury results in neuronal cell degeneration.
- 30 30. The method of claim 23, wherein the disease is selected from the group consisting of Huntington's disease, Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, peripheral neuropathy, spinal

muscular atrophy, Creutzfeldt-Jakob disease, AIDS dementia, progressive supranuclear palsy, myelinopathia centralis diffusa (vanishing white matter disease), chronic neurodegenerative disease, Down's syndrome, leukoencephalopathy and Schilder's disease.

5

31. The method of claim 23, wherein the injury or disease is a result of one or more conditions selected from the group consisting of neuroblastoma, head injury, traumatic brain injury, stroke, ischemic injury, hypoxic injury, reperfusion injury, epilepsy, cardiac artery bypass graft surgery, toxin damage,
10 radiation damage and asphyxia.

32. The method of claim 23, wherein the injury or disease is a result of an inflammatory condition.

15 33. The method of claim 23, wherein the injury or disease is a result of one or more conditions selected from the group consisting of chronic or acute encephalomyelitis, encephalitis, optic neuritis, transverse myelitis, meningitis, panencephalitis, Devic's disease, progressive multifocal leukoencephalopathy, central pontine myelinolysis and neuromyelitis optica.

20

34. The method of claim 23, wherein the disease is a result of schizophrenia or depression.

25 35. A method of claim 23, wherein at least one other anti-apoptotic, anti-necrotic or neuroprotective agent is administered.

36. The method of claim 35 where the other anti-apoptotic or neuroprotective agent is selected from selected from growth factors and associated derivatives (insulin-like growth factor-I [IGF-I], insulin-like growth
30 factor-II [IGF-II], transforming growth factor- β 1, activin, growth hormone,

nerve growth factor, growth hormone binding protein, IGF-binding proteins [especially IGFBP-3], basic fibroblast growth factor, acidic fibroblast growth factor, the hst/Kfgk gene product, FGF-3, FGF-4, FGF-6, keratinocyte growth factor, androgen-induced growth factor, int-2, fibroblast growth factor
5 homologous factor-1 (FHF-1), FHF-2, FHF-3 and FHF-4, keratinocyte growth factor 2, glial-activating factor, FGF-10 and FGF-16, ciliary neurotrophic factor, brain derived growth factor, neurotrophin 3, neurotrophin 4, bone morphogenetic protein 2 [BMP-2], glial-cell line derived neurotrophic factor, activity-dependant neurotrophic factor, cytokine leukaemia inhibiting factor,
10 oncostatin M, an interleukin, α - interferon, β - interferon, γ - interferon, consensus interferon, TNF- α , clomethiazole; kynurenic acid, Semax, tacrolimus, L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, adrenocorticotropin-(4-9) analogue [ORG 2766], dizolcipine [MK-801], selegiline, a glutamate antagonist, an AMPA antagonist and an anti-
15 inflammatory agent.

37. The method of claim 36 wherein said glutamate antagonist is selected from the group consisting of NPS1506, GV1505260, MK-801 and GV150526.

20 38. The method of claim 36 wherein said AMPA antagonist is selected from the group consisting of 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX), LY303070 and LY300164.

25 39. The method of claim 36, wherein said anti-inflammatory agent is selected from the group consisting of an anti-MAdCAM-1 antibody and an antibody against an integrin $\alpha 4 \beta 1$ receptor and an integrin $\alpha 4 \beta 7$ receptor.

40. The method of claim 39 wherein said anti-MAdCAM-1 antibody is MECA-367.

30

41. The method of claim 23 where the compound is (2S, 3'S, 8'R, 11'S) 2-
{[(3'-Amino-1'-aza -2'-oxobicyclo[6.3.0]-undecyl)-11'-carbonyl]amino}-1,5-
pentanedioic acid trifluoroacetate salt (48).
- 5 42. The method of claim 23 where the compound is (2S, 9'R, 12'S)-2-
{[(1',4'-Diaza-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino}-1,5-
pentanedioic acid trifluoroacetate (68).
43. The method of claim 23, wherein said condition is hypoxic eschemia.
- 10 44. The method of claim 23, wherein said condition is neurotoxicity.

P7-cerebellar microexplants
injured by 0.5mM
3NP/glutamate and rescued by
(2S, 3'S, 8'R, 11'S) 2-[(3'-Amino-1'-aza-2'-oxobicyclo[6.3.0]undecyl)-
-11'-carbonyl]amino)-1,5-pentanedioic acid trifluoroacetate salt (48)

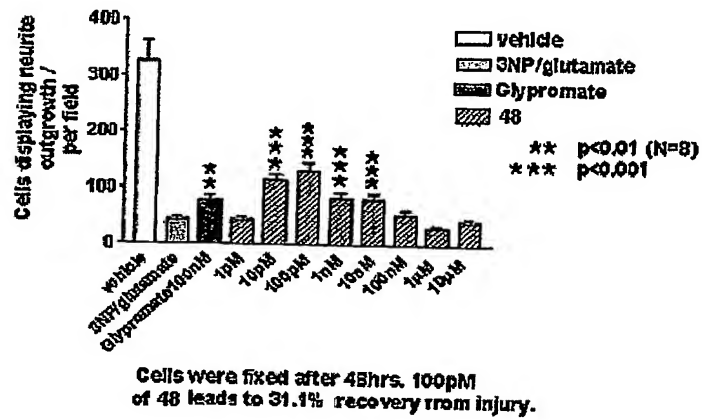


Figure 1

Cerebellar microexplants
injured by 0.5mM
3-NP/glutamate and rescued by
(2S, 9'R, 12'S)-2-[[[1',4'-Diaz-2'-oxobicyclo[7.3.0]dodecyl)-12'-carbonyl]amino]-
-1,5-pentanedioic acid trifluoroacetate (68)

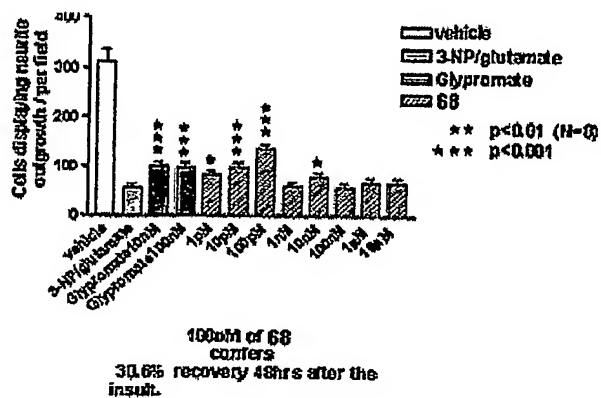


Figure 2

100 pM 68 given i.c.v. 2 h after HI injury

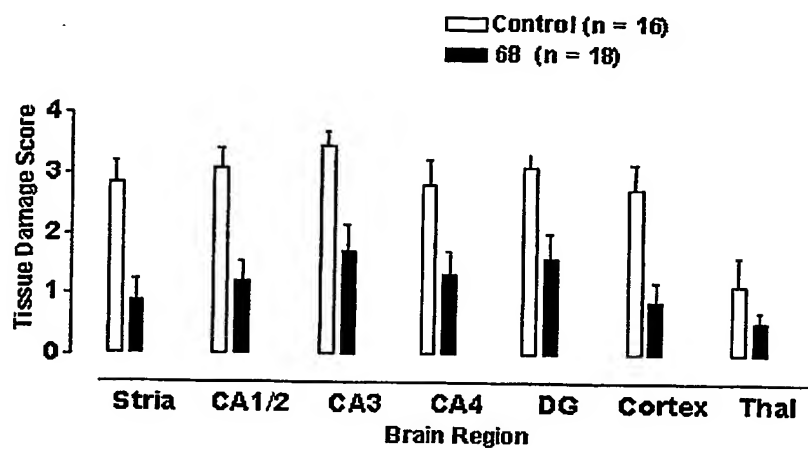


Figure 3

Rec'd PCT/PTO 20 SEP 2005

10/549951

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
7 October 2004 (07.10.2004)

PCT

(10) International Publication Number
WO 2004/084809 A3

(51) International Patent Classification⁷: C07D 225/00,
245/04, A61K 31/395, A61P 25/16, 25/28

(21) International Application Number:
PCT/US2004/008108

(22) International Filing Date: 16 March 2004 (16.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/456,136 20 March 2003 (20.03.2003) US
60/505,119 23 September 2003 (23.09.2003) US

(71) Applicants (for all designated States except US):
NEURONZ LIMITED [NZ/NZ]; Level 3, 2-6 Park
Avenue, Grafton, Auckland 1030 (NZ). NEURONZ
BIOSCIENCES, INC. [US/US]; 2711 Centerville Road -
Suite 400, Wilmington, De 19801 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): HARRIS, Paul, W.,
R. [NZ/NZ]; 111 Christian Road, Swansan, Auckland 1030
(NZ). BRIMBLE, Margaret, Anne [NZ/NZ]; 11 A Tor-
rance Street, Epsom, Auckland 1003 (NZ).

(74) Agent: BORSON, D., Benjamin; Fliesler Meyer LLP,
Four Embarcadero Center, Fourth Floor, San Francisco, CA
94111-4156 (US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Euro-
pean (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR,
GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(88) Date of publication of the international search report:
30 June 2005

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: NEUROPROTECTIVE MACROCYCLIC COMPOUNDS AND METHODS FOR THEIR USE

(57) Abstract: Embodiments of this invention provide novel peptidomimetics that contain a macrocycle. Such compounds are neu-
roprotective and have utility as therapeutic agents for treatment of diseases, injuries and other conditions characterised by neuronal
degeneration and/or death. Compounds are also useful for manufacture of medicaments useful for treatment of such conditions.

WO 2004/084809 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/08108

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 225/00, 245/04; A61K 31/395; A61P 25/16, 25/28
US CL : 540/460, 461; 514/412

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 540/460, 461; 514/412

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BEVISI et al., European Journal of Organic Chemistry, 1999, 289-400.	1-6

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

21 April 2005 (21.04.2005)

Date of mailing of the international search report

11 MAY 2005

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Bruck Kifle, Ph.D.
Telephone No. 571-272-0668